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PLANT PHYSIOLOGY

JULY, 1948

MINERAL ACCUMULATION IN CORN

J. D. SAYRE[1]

(WITH FOUR FIGURES)

Received August 23, 1947

Introduction

This study is the result of attempts to follow the growth and development of corn in relation to the accumulation and movement of the mineral elements in the plants. Three years' work was carried out on the subject at Wooster, Ohio, but only the last year's data have been carefully studied and analyzed. The double cross, Ohio K35, was used in the study and only the three major elements, nitrogen, phosphorus, and potassium, have been summarized.

A great deal of research has been done with corn on such subjects as feed analysis, fertilizers, culture, breeding, and genetics, but little has been done on seasonal development, rate of growth, or mineral accumulation. SCHWEITZER (**7**) reported the green and the dry weight per plant and feed analysis on one to five plant samples taken every five or six days. The work was done in 1888 on "St. Charles White" at the Missouri Agricultural Experiment Station. JONES AND HUSTON (**4**) reported similar data for "Riley's Favorite" grown in 1903. The plants were taken at seven stages of growth and each sample included 10 to 15 plants. Nitrogen, phosphorus, and potassium were reported on the samples in addition to feed analyses. HOPPER (**3**) grew eighteen varieties of corn in 1920 in North Dakota and sampled 60 plants of each variety at five stages of growth after tasseling. He reported dry matter determinations and feed analyses on the samples. DULEY AND MILLER (**2**) grew "Reid's Yellow Dent" in nutrient cultures in 1913 to 1916. They harvested 3 plants from each treatment at 30, 60, and 90 days after planting and published the results in 1921. They gave dry weights and mineral analysis of the samples. MILLER (**5**) grew "Kansas Sunflower" corn in 1924 to 1927 and obtained dry weights of plant parts every week on 5 plant samples for each of the four years.

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Experimental methods

METHODS OF FIELD SAMPLING. The corn used in this experiment was grown in rows 42 inches apart with the plants spaced 14 inches apart in the rows. Every other row was left as a guard row in order to provide normal competition. The samples consisted of four units of five consecutive plants each, duplicated. Before sampling began, the units were marked with small pot labels, leaving two extra plants between each unit, and after proper numbering a random drawing of all samples for the entire summer was made. The first samples were taken one month after planting. Only units having a full stand were selected. Extra units were provided to replace plants that were lost as the season progressed. Samples were taken every three days.

DISSECTION OF THE PLANTS. The plants were cut at the surface of the soil and brought into the laboratory. The height or length was measured and the plants dissected into the different tissues. When the plants were small, no separation was made but later the leaves were removed leaving the sheath with the stem. Still later the ear was separated from the stem and finally the grain, cob, and husks (including shank) handled individually. It was necessary to dissect the corn plants in this manner for two reasons. First, it is impossible to obtain a truly representative sample of a full-sized corn plant by cutting up whole plants because of differences in the water content of the various tissues, differences in type and density of tissues and other factors which may cause separation or sedimentation of the finely cut plants. It is also necessary to have an analysis of each tissue separately if studies of growth and development are to be made, accompanied by measurements of accumulation and movement of mineral elements from one tissue to another.

DRY MATTER DETERMINATIONS. After sorting out each kind of tissue, it was placed in a large container and the green weight determined just before grinding. The samples were run through a food cutter if they were not too large and, after thoroughly mixing, a representative sub-sample of 100 or 200 grams was dried to constant weight at 70° C. in a forced air oven. The very large samples were passed through a small silage cutter first, one gallon of the cut material was then run through the food cutter, and a sub-sample removed for drying. In some instances, all the tissue was dried and kept for analysis.

PREPARATION FOR CHEMICAL ANALYSIS. All samples were stored in paper bags or cardboard cartons for mineral analysis. The final grinding depended on the type and amount of the sample available. An intermediate Wiley mill, a micro-pulverizer, a Krupp mill, a large Wiley mill, and a large hammer mill were available to reduce the cut or chopped sample to fine powder. About 10 grams of the final sample were kept in tightly stoppered bottles until analysis.

CHEMICAL ANALYSIS. Total nitrogen was determined by the Kjeldahl procedure (1). Total phosphorus was determined on a two-gram sample of

the tissue after wet digestion with nitric and perchloric acid by Zinzade's method (**8**). Potassium was determined on the same wet digestion sample by a modification of the cobaltinitrite method (**6**). The results have been calculated on a dry weight basis as total amount of each element in the different tissues and the total amount per plant. All data have been smoothed by moving averages, using a three-unit average and three moves. The data for the whole plant are the sums of the moving-average values of each tissue.

Experimental results

DRY MATTER PRODUCTION. The dry matter data for 1940 are given in table I. The gain or loss for each three-day period is given. The first values on June 20 were taken one month after planting.

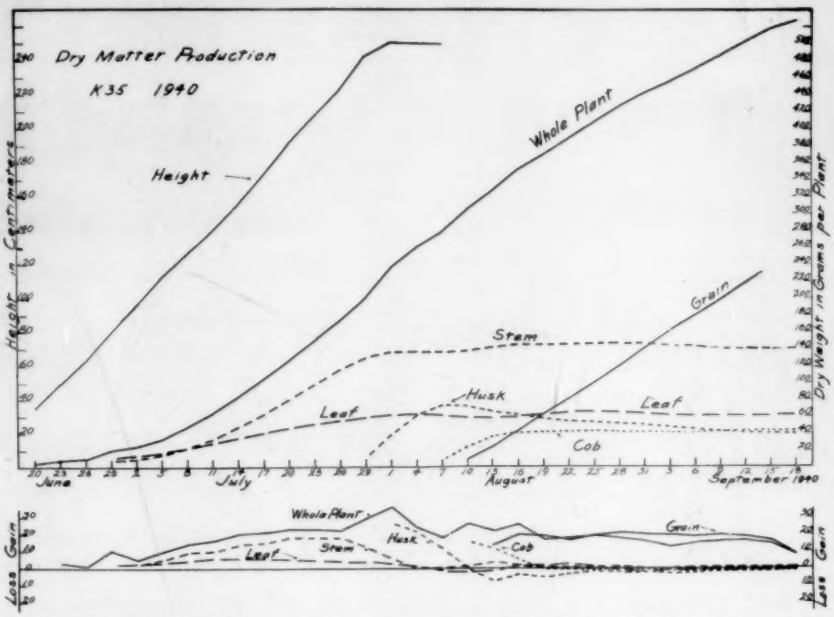

FIG. 1. Graphs of dry matter production per plant of the different tissues of K35 grown at Wooster, Ohio, 1940.

The maximum rate of dry matter production occurred between July 26 and August 4 during which time tasseling and silking had taken place and growth in height had ceased. The maximum weight of leaves and stems was reached about August 22, although maximum leaf area and height had occurred earlier. The first ears were obtained July 29, but they had to be handled as a unit, as grain could not be separated from them until later. The data given under husks in table I for July 29 to August 4 included the entire young ear. On August 7 husks and ears were separated, but no grain was taken from the ear. Separate values for husks, cob, and grain were obtained on August 10. After grain formation started, the increase in weight of the whole plant was only slightly less than that of grain for-

TABLE I

WEIGHT PER PLANT OF DRY MATTER IN GRAMS. K35, WOOSTER, OHIO, 1940

DATE	LEAF		STEM		HUSK		COB		GRAIN		WHOLE PLANT	
	WEIGHT	GAIN OR LOSS	WEIGHT	GAIN OR LOSS	WEIGHT	GAIN OR LOSS	WEIGHT	GAIN OR LOSS	WEIGHT	GAIN OR LOSS	WEIGHT	GAIN OR LOSS
June 20	5										5	
23	8	+3									8	+ 3
26	9	+1									9	+ 1
29	11	+2	8								19	+10
July 2	14	+3	10	+ 2							24	+ 5
5	18	+4	15	+ 5							33	+ 9
8	23	+5	24	+ 9							47	+14
11	29	+6	34	+10							63	+16
14	35	+6	48	+14							83	+20
17	41	+6	63	+15							104	+21
20	46	+5	81	+18							127	+23
23	51	+5	99	+18							150	+23
26	55	+4	117	+18							172	+22
29	59	+4	130	+13	12						201	+29
Aug. 1	62	+3	136	+ 6	39	+27					237	+36
4	63	+1	137	+ 1	61	+22					261	+24
7	61	−2	137	0	73	+12	8				279	+18
10	59	−2	139	+ 2	73	0	24	+16	10		305	+26
13	58	−1	143	+ 4	66	− 7	36	+12	24	+14	327	+22
16	60	+2	145	+ 2	63	− 3	41	+ 5	44	+20	353	+26
19	62	+2	145	0	58	− 5	42	+ 1	63	+19	370	+17
22	65	+3	145	0	55	− 3	43	+ 1	80	+17	388	+18
25	66	+1	146	+ 1	54	− 1	43	0	99	+19	408	+20
28	65	−1	147	+ 1	52	− 2	42	− 1	120	+21	426	+18
31	63	−2	147	0	50	− 2	42	0	140	+20	442	+16
Sept. 3	62	−1	144	− 3	47	− 3	42	0	160	+20	455	+13
6	61	−1	143	− 1	45	− 2	42	0	179	+19	470	+15
9	61	0	141	− 2	43	− 2	42	0	199	+20	486	+16
12	61	0	140	− 1	42	− 1	42	0	218	+19	503	+17
15	61	0	140	0	40	− 2	42	0	235	+17	518	+15
18	61	0	140	0	40	0	42	0	243	+ 8	526	+ 8

mation. All the dry matter produced in photosynthesis passed into the grain with only a little loss from the other tissues of the plant except the husks.

The data are summarized in table I and presented graphically in figure 1. The first point on the graphs indicates a date one month after planting. The height growth values are shown in this figure for comparison with dry weight determinations. Height growth was practically a straight line function of time with no indication of a sigmoid type of growth. Height growth ended abruptly and decreased slightly due to the loss of leaf tips. The dry weight curve of the whole plant had a slight tendency to be sig-

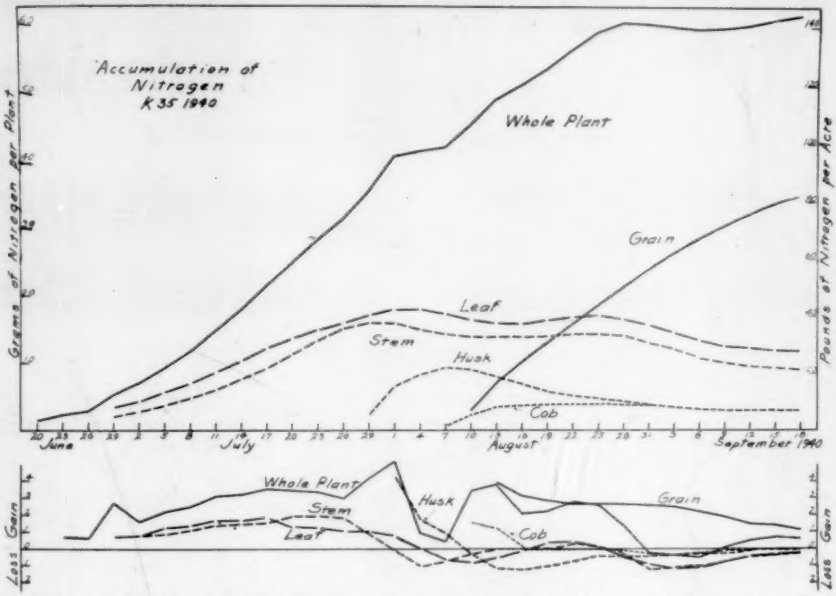

FIG. 2. Graphic representation of nitrogen accumulation per plant and its movement in K35 grown at Wooster, Ohio, 1940.

moid in character, but none of the other graphs, with the exception of the grain, exhibited this tendency. The curves for the gain or loss of the different parts of the plant show quite clearly when dry matter production ceased and losses occurred. They cannot be described by any mathematical formula. The hump in the curve for husks is due to the fact that both cob and grain were included in the first few samples.

NITROGEN ACCUMULATION. Table II gives the values for nitrogen accumulation in the different tissues of the plant including the gain or loss for each three-day period. Only the total amount in the tissue is given, this having been calculated from the percentage composition. The trends in nitrogen accumulation are similar, though not identical, to dry matter production.

The accumulation of nitrogen continued until August 28 after which minor fluctuations occurred, with no indication of a continued rise as was

TABLE II

ACCUMULATION PER PLANT OF NITROGEN IN GRAMS. K35, WOOSTER, OHIO, 1940

DATE	LEAF		STEM		HUSK		COB		GRAIN		WHOLE PLANT	
	WEIGHT	GAIN OR LOSS	WEIGHT	GAIN OR LOSS	WEIGHT	GAIN OR LOSS	WEIGHT	GAIN OR LOSS	WEIGHT	GAIN OR LOSS	WEIGHT	GAIN OR LOSS
June 20	0.15										0.15	
23	0.22	+0.07									0.22	+0.07
26	0.28	+0.06									0.28	+0.06
29	0.35	+0.07	0.20								0.55	+0.27
July 2	0.43	+0.08	0.28	+0.08							0.71	+0.16
5	0.56	+0.13	0.37	+0.09							0.93	+0.22
8	0.70	+0.14	0.48	+0.11							1.18	+0.25
11	0.87	+0.17	0.62	+0.14							1.49	+0.31
14	1.04	+0.17	0.77	+0.15							1.81	+0.32
17	1.23	+0.19	0.93	+0.16							2.16	+0.35
20	1.37	+0.14	1.13	+0.20							2.50	+0.34
23	1.50	+0.13	1.33	+0.20							2.83	+0.33
26	1.61	+0.11	1.52	+0.19							3.13	+0.30
29	1.72	+0.11	1.61	+0.09	0.22						3.55	+0.42
Aug. 1	1.80	+0.08	1.61	0.0	0.66	+0.44					4.07	+0.52
4	1.81	+0.01	1.51	−0.10	0.84	+0.18					4.16	+0.09
7	1.75	−0.06	1.44	−0.07	0.94	+0.10	0.08				4.21	+0.05
10	1.67	−0.08	1.41	−0.03	0.92	−0.02	0.24	+0.16	0.31		4.55	+0.34
13	1.62	−0.05	1.41	0.0	0.81	−0.11	0.37	+0.13	0.71	+0.40	4.92	+0.37
16	1.61	−0.01	1.41	0.0	0.69	−0.12	0.39	+0.02	1.03	+0.32	5.13	+0.21
19	1.65	+0.04	1.41	0.0	0.59	−0.10	0.39	0.0	1.32	+0.29	5.36	+0.23
22	1.70	+0.05	1.44	+0.03	0.52	−0.07	0.39	0.0	1.59	+0.27	5.64	+0.28
25	1.72	+0.02	1.45	+0.01	0.48	−0.04	0.39	0.0	1.86	+0.27	5.90	+0.26
28	1.67	−0.05	1.42	−0.03	0.44	−0.04	0.38	−0.01	2.13	+0.27	6.04	+0.14
31	1.58	−0.09	1.30	−0.12	0.39	−0.05	0.36	−0.02	2.39	+0.26	6.02	−0.02
Sept. 3	1.46	−0.12	1.19	−0.11	0.36	−0.03	0.33	−0.03	2.64	+0.25	5.98	−0.04
6	1.35	−0.11	1.08	−0.11	0.33	−0.03	0.31	−0.02	2.86	+0.22	5.93	−0.05
9	1.27	−0.08	1.01	−0.07	0.31	−0.02	0.30	−0.01	3.05	+0.19	5.94	+0.01
12	1.23	−0.04	0.96	−0.05	0.30	−0.01	0.30	0.0	3.20	+0.15	5.99	+0.05
15	1.20	−0.03	0.93	−0.03	0.30	0.0	0.29	−0.01	3.34	+0.14	6.06	+0.07
18	1.18	−0.02	0.90	−0.03	0.30	0.0	0.29	0.0	3.45	+0.11	6.12	+0.06

evident in dry matter values. But the nitrogen continued to accumulate in the grain as long as the plants were sampled. This indicates that nitrogen moved from the other tissues of the plant into the grain. Some nitrogen moved out of the cob, a considerable amount from the husks, and some from the stem and leaves. The greatest amount of nitrogen had accumulated in the plant tissues, with the exception of the grain, about the first of August. The greatest rate of accumulation also occurred about this time, which was about a week later than the time of maximum dry matter production.

Figure 2 presents the results graphically of the nitrogen accumulation in the tissues of the plant. This graph indicates very clearly that total

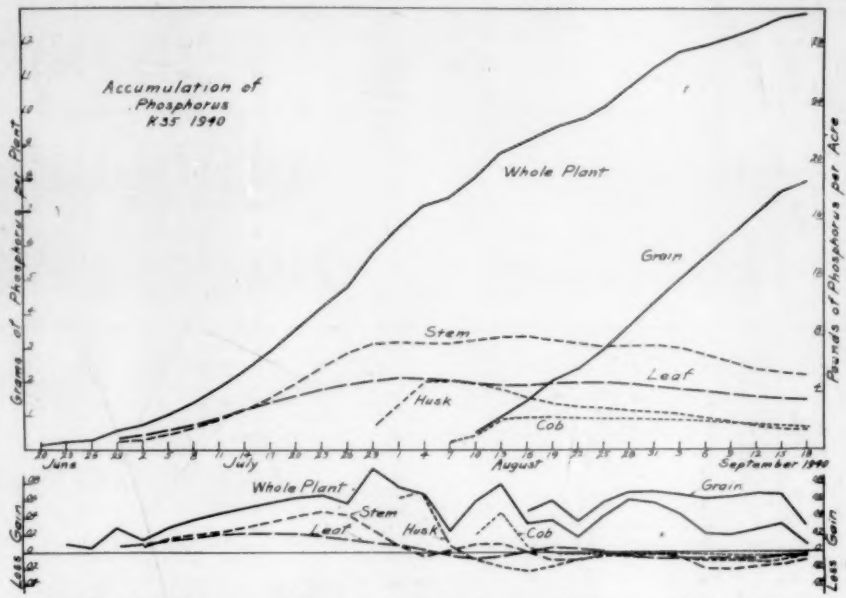

FIG. 3. Graphic representation of phosphorus accumulation per plant and its movement in K35 grown at Wooster, Ohio, 1940.

accumulation stopped about August 28. The grain continued to increase in nitrogen until the end of the experiment and the husks lost a great deal of nitrogen after the grain began to form.

PHOSPHORUS ACCUMULATION. Table III lists the values for phosphorus accumulation. In many ways these data are similar to those for nitrogen accumulation. The maximum amounts were present in the leaves, stems, and husks at the same time as was the nitrogen, and the grain continued to increase as long as it was sampled. The maximum rate of phosphorus accumulation occurred at the same period as nitrogen, but the total amount per plant continued to increase as long as the plants were sampled. This shows that the plant continued to absorb phosphorus from the soil all during the season, since the loss which occurred from the leaves, stem, husks, and cob did not account for the quantity which moved into the grain.

TABLE III

ACCUMULATION PER PLANT OF PHOSPHORUS IN GRAMS. K35, WOOSTER, OHIO, 1940

DATE	LEAF		STEM		HUSK		COB		GRAIN		WHOLE PLANT	
	WEIGHT	GAIN OR LOSS	WEIGHT	GAIN OR LOSS	WEIGHT	GAIN OR LOSS	WEIGHT	GAIN OR LOSS	WEIGHT	GAIN OR LOSS	WEIGHT	GAIN OR LOSS
June 20	0.013										0.013	
23	0.023	+0.010									0.023	+0.010
26	0.028	+0.005									0.028	+0.005
29	0.034	+0.006	0.023								0.057	+0.029
July 2	0.043	+0.009	0.029	+0.006							0.072	+0.015
5	0.057	+0.014	0.044	+0.015							0.101	+0.029
8	0.074	+0.017	0.065	+0.021							0.139	+0.038
11	0.095	+0.021	0.089	+0.024							0.184	+0.045
14	0.116	+0.021	0.118	+0.029							0.234	+0.050
17	0.138	+0.022	0.153	+0.035							0.291	+0.057
20	0.159	+0.021	0.195	+0.042							0.354	+0.063
23	0.177	+0.018	0.242	+0.047							0.419	+0.065
26	0.191	+0.014	0.285	+0.043							0.476	+0.057
29	0.201	+0.010	0.311	+0.026	0.063						0.575	+0.099
Aug. 1	0.208	+0.007	0.317	+0.006	0.126	+0.063					0.651	+0.076
4	0.209	+0.001	0.312	−0.005	0.197	+0.071					0.718	+0.067
7	0.204	−0.005	0.314	+0.002	0.204	+0.007	0.020				0.742	+0.024
10	0.196	−0.008	0.324	+0.010	0.194	−0.010	0.040	+0.020	0.047		0.801	+0.059
13	0.190	−0.006	0.334	+0.010	0.176	−0.018	0.086	+0.046	0.094	+0.047	0.880	+0.079
16	0.190	0.0	0.334	0.0	0.153	−0.023	0.094	+0.008	0.142	+0.048	0.913	+0.033
19	0.195	+0.005	0.324	−0.010	0.135	−0.018	0.094	0.0	0.202	+0.060	0.950	+0.037
22	0.199	+0.004	0.314	−0.010	0.125	−0.010	0.092	−0.002	0.238	+0.036	0.968	+0.018
25	0.199	0.0	0.307	−0.007	0.120	−0.005	0.089	−0.003	0.295	+0.057	1.010	+0.042
28	0.194	−0.005	0.308	+0.001	0.115	−0.005	0.088	−0.001	0.365	+0.070	1.070	+0.060
31	0.187	−0.007	0.308	0.0	0.109	−0.006	0.087	−0.001	0.436	+0.071	1.127	+0.057
Sept. 3	0.179	−0.008	0.302	−0.006	0.102	−0.007	0.085	−0.002	0.503	+0.067	1.171	+0.044
6	0.171	−0.008	0.282	−0.020	0.093	−0.009	0.081	−0.004	0.565	+0.062	1.192	+0.021
9	0.163	−0.008	0.261	−0.021	0.082	−0.011	0.077	−0.004	0.629	+0.064	1.212	+0.020
12	0.155	−0.008	0.243	−0.018	0.070	−0.012	0.073	−0.004	0.697	+0.068	1.238	+0.026
15	0.150	−0.005	0.228	−0.015	0.060	−0.010	0.070	−0.003	0.764	+0.067	1.272	+0.034
18	0.145	−0.005	0.218	−0.010	0.059	−0.001	0.067	−0.003	0.793	+0.029	1.282	+0.010

The results of phosphorus accumulation are shown in graphic form in figure 3. The continued rise in phosphorus accumulation in the whole plant is clearly shown by these data, as is the rapid accumulation in the grain. The amount of phosphorus which moved into the grain is practically a straight line function with time.

POTASSIUM ACCUMULATION. Table IV shows the data for potassium accumulation in the plant. These data differ from those of the other two elements in several ways. The grain does not accumulate much potassium. The maximum rate of accumulation and the time when the maximum amount occurs in the leaves and stems is earlier than for nitrogen and phosphorus. There is a small but consistent increase in the amount of

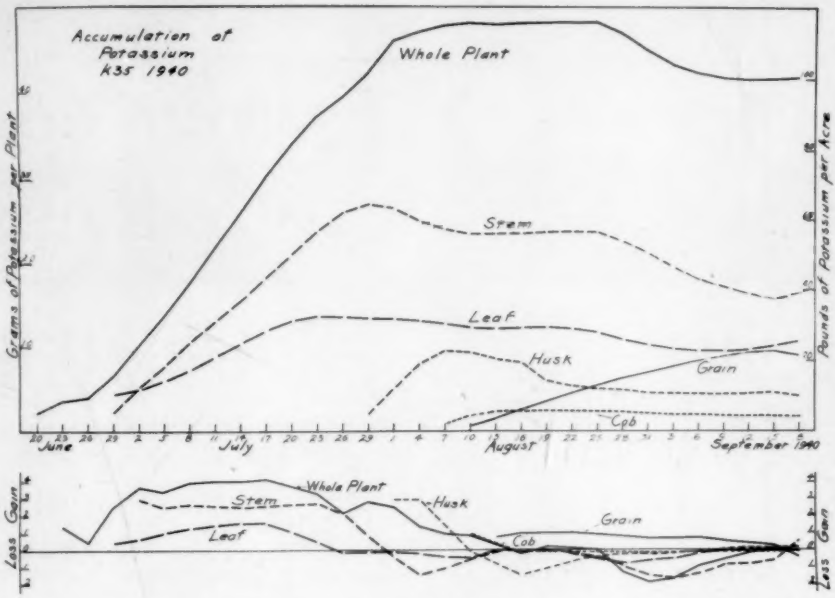

FIG. 4. Graphic representation of potassium accumulation per plant and its movement in K35 grown at Wooster, Ohio, 1940.

potassium in the grain and a rather marked loss of potassium from the other plant tissues, especially the stem. The plant as a whole loses potassium after August 25. In other words, the amount of potassium in the plant reaches a maximum about that date and then decreases. This behavior is quite different than that for the other two elements.

Figure 4 shows the potassium data in graphic form. The loss from the plant after August 25 is clearly shown. The small amount of potassium contained in the grain in comparison to the other two elements, and the fact that half of it is concentrated in the stem tissue, is shown in figure 4.

ACCUMULATION IN POUNDS PER ACRE. The pounds per acre of dry matter, and a summation of the data in the previous tables appear in table V. During the grand period of growth from July 20 to August 20, almost

TABLE IV

ACCUMULATION PER PLANT OF POTASSIUM IN GRAMS. K35, WOOSTER, OHIO, 1940

DATE	LEAF		STEM		HUSK		COB		GRAIN		WHOLE PLANT	
	WEIGHT	GAIN OR LOSS	WEIGHT	GAIN OR LOSS	WEIGHT	GAIN OR LOSS	WEIGHT	GAIN OR LOSS	WEIGHT	GAIN OR LOSS	WEIGHT	GAIN OR LOSS
June 20	0.21										0.21	
23	0.35	+0.14									0.35	+0.14
26	0.39	+0.04									0.39	+0.04
29	0.43	+0.04	0.22								0.65	+0.26
July 2	0.50	+0.07	0.52	+0.30							1.02	+0.37
5	0.60	+0.10	0.77	+0.25							1.37	+0.35
8	0.73	+0.13	1.04	+0.27							1.77	+0.40
11	0.88	+0.15	1.30	+0.26							2.18	+0.41
14	1.04	+0.16	1.55	+0.25							2.59	+0.41
17	1.20	+0.16	1.81	+0.26							3.01	+0.42
20	1.31	+0.11	2.08	+0.27							3.39	+0.38
23	1.36	+0.05	2.36	+0.28							3.72	+0.33
26	1.35	−0.01	2.59	+0.23							3.94	+0.22
29	1.34	−0.01	2.68	+0.09	0.20						4.22	+0.28
Aug. 1	1.33	−0.01	2.64	−0.04	0.50	+0.30					4.47	+0.25
4	1.31	−0.02	2.50	−0.14	0.80	+0.30					4.61	+0.14
7	1.27	−0.04	2.39	−0.11	0.95	+0.15	0.10				4.71	+0.10
10	1.23	−0.04	2.34	−0.05	0.94	−0.01	0.20	+0.10	0.08		4.79	+0.08
13	1.22	−0.01	2.34	0.0	0.86	−0.08	0.24	+0.04	0.16	+0.08	4.82	+0.03
16	1.23	+0.01	2.34	0.0	0.72	−0.14	0.25	+0.01	0.26	+0.10	4.80	−0.02
19	1.24	+0.01	2.36	+0.02	0.61	−0.11	0.25	0.0	0.36	+0.10	4.82	+0.02
22	1.22	−0.02	2.36	0.0	0.54	−0.07	0.25	0.0	0.46	+0.10	4.83	+0.01
25	1.17	−0.05	2.36	0.0	0.51	−0.03	0.24	−0.01	0.55	+0.09	4.83	0.0
28	1.09	−0.08	2.26	−0.10	0.49	−0.02	0.23	−0.01	0.63	+0.08	4.70	−0.13
31	1.03	−0.06	2.11	−0.15	0.46	−0.03	0.21	−0.02	0.70	+0.07	4.51	−0.19
Sept. 3	0.98	−0.05	1.94	−0.17	0.45	−0.01	0.20	−0.01	0.77	+0.07	4.34	−0.17
6	0.96	−0.02	1.80	−0.14	0.44	−0.01	0.20	0.0	0.84	+0.07	4.24	−0.10
9	0.95	−0.01	1.71	−0.09	0.44	0.0	0.19	−0.01	0.89	+0.05	4.18	−0.06
12	0.97	+0.02	1.63	−0.08	0.45	+0.01	0.18	−0.01	0.93	+0.04	4.16	−0.02
15	1.01	+0.04	1.57	−0.06	0.46	+0.01	0.18	0.0	0.95	+0.02	4.17	+0.01
18	1.06	+0.05	1.63	+0.06	0.42	−0.04	0.17	−0.01	0.90	−0.05	4.18	+0.01

three tons of dry matter were produced per acre. This represents an accumulation of carbon dioxide and water synthesized to carbohydrates together with some 67 pounds of nitrogen, 14 pounds of phosphorus, and 34 pounds of potassium.

TABLE V

TOTAL ACCUMULATION IN POUNDS PER ACRE. K35, WOOSTER, OHIO, 1940

DATE	DRY MATTER		NITROGEN		PHOSPHORUS		POTASSIUM	
	WEIGHT	GAIN OR LOSS	WEIGHT	GAIN OR LOSS	WEIGHT	GAIN OR LOSS	WEIGHT	GAIN OR LOSS
June 20	118		3.5		0.3		4.9	
23	188	+ 70	5.2	+ 1.7	0.5	+0.2	8.2	+3.3
26	212	+ 24	6.6	+ 1.4	0.7	+0.2	9.2	+1.0
29	447	+235	13.0	+ 6.4	1.3	+0.6	15.3	+6.1
July 2	565	+118	16.7	+ 3.7	1.7	+0.4	24.0	+8.7
5	777	+212	21.9	+ 5.2	2.4	+0.7	32.3	+8.3
8	1107	+330	27.8	+ 5.9	3.3	+0.9	41.7	+9.4
11	1484	+377	35.1	+ 7.3	4.3	+1.0	51.3	+9.6
14	1955	+471	42.6	+ 7.5	5.5	+1.2	61.0	+9.7
17	2449	+494	50.9	+ 8.3	6.9	+1.4	70.9	+9.9
20	2991	+542	58.9	+ 8.0	8.3	+1.4	79.8	+8.9
23	3533	+542	66.6	+ 7.7	9.9	+1.6	87.6	+7.8
26	4051	+518	73.7	+ 7.1	11.2	+1.3	92.8	+5.2
29	4734	+683	83.6	+ 9.9	13.5	+2.3	99.4	+6.6
Aug. 1	5581	+847	95.8	+12.2	15.3	+1.8	105.3	+5.9
4	6147	+566	98.0	+ 2.2	16.9	+1.6	108.6	+3.3
7	6570	+423	99.1	+ 1.1	17.5	+0.6	110.9	+2.3
10	7183	+613	107.2	+ 8.1	18.9	+1.4	112.8	+1.9
13	7701	+518	115.9	+ 8.7	20.7	+1.8	113.5	+0.7
16	8313	+612	120.8	+ 4.9	21.5	+0.8	113.0	−0.5
19	8714	+401	126.2	+ 5.4	22.4	+0.9	113.5	+0.5
22	9137	+423	132.8	+ 6.6	22.8	+0.4	113.7	+0.2
25	9608	+471	138.9	+ 6.1	23.8	+1.0	113.7	0.0
28	10032	+424	142.2	+ 3.3	25.2	+1.4	110.7	−3.0
31	10409	+377	141.8	− 0.4	26.5	+1.3	106.2	−4.5
Sept. 3	10715	+306	140.8	− 1.0	27.6	+1.1	102.2	−4.0
6	11069	+354	139.7	− 1.1	28.1	+0.5	99.9	−2.3
9	11445	+376	139.9	+ 0.2	28.5	+0.4	98.4	−1.5
12	11846	+401	141.1	+ 1.2	29.2	+0.7	98.0	−0.4
15	12199	+353	142.7	+ 1.6	30.0	+0.8	98.2	+0.2
18	12387	+188	144.1	+ 1.4	30.2	+0.2	98.4	+0.2

The maximum dry weights of plant parts in pounds per acre are shown below:

	Leaves	Stems	Husks	Cobs	Grain
Grams	65	147	50	42	243
Pounds	1531	3462	1179	989	5723

The calculated yield per acre for a grain weight of 243 grams per plant would be over 100 bushels of shelled grain. Starter nitrogen amounted to 3.5 pounds per acre, if we consider the one-month-old plants to be started. Ten days later, on July 1, they had accumulated 15 pounds per acre. The

greatest accumulation of nitrogen occurred the last few days in July, when the daily intake was 4.0 pounds per acre. From July 11 to August 4 the daily accumulation averaged 2.7 pounds per acre. Total accumulation stopped suddenly on August 28, and a slight loss occurred after that date, probably due to the loss of some of the leaf tissues by shattering.

The fact that plants accumulate relatively small amounts of phosphorus is shown in table V. However, phosphorus, even though in these small amounts, is just as necessary and important as nitrogen. The maximum rate of potassium accumulation occurred during the period from July 5 to July 17, when an average of 3.2 pounds per acre entered the plant. In contrast to the other two elements, there was a loss of potassium from the plant amounting to about 16 pounds per acre.

Discussion of results

During the first month after planting, the seeds germinated, and the plants grew to a total dry weight of about five grams each. The entire plant tissue was considered as leaf tissue until it became possible to separate the stem from the leaves. By July 26, tasseling had begun and it was possible to separate the young ear from the stem on July 29. Soon thereafter it was possible to separate the husks from the ears and finally, on August 10, grain was separated from the cob. From this time on, grain formation and carbohydrate movement into the ear were the only growth processes occurring. This only became evident when the total dry weight of the plants was measured. At silking, only about half the final dry weight of the plant had been produced; that is, the plants were half-grown, although their full height had been attained.

An attempt has been made in this discussion to study the accumulation and movement of the three major elements in the different tissues of the plant. In order to find out when the greatest period of accumulation occurred, the data have been divided into four groups of 30 days each, beginning with planting on May 20. The first period ends on June 20, the second on July 20, the third on August 19, and the fourth period on the last day of sampling, September 18. The following tabulation shows the results of this study (table VI).

TABLE VI

DRY WEIGHT AND MINERAL ACCUMULATION BY FOUR 30-DAY PERIODS STARTING AT PLANTING TIME ON MAY 20

PERIOD	DRY WT.		NITROGEN		PHOSPHORUS		POTASSIUM	
	gm.	%	*gm.*	%	*gm.*	%	*gm.*	%
May 20 to June 20	5	1	0.15	2	0.013	1	0.21	4
June 20 to July 20	122	23	2.35	38	0.341	27	3.18	66
July 20 to Aug. 19	243	46	2.86	47	0.596	46	1.43	30
Aug. 19 to Sept. 18	156	30	0.76	12	0.332	26	0.00	0
Total	526		6.12		1.282		4.82	

About half the dry weight was produced during the third 30-day period. The greatest nitrogen and phosphorus accumulation occurred during this same period, but the greatest potassium accumulation occurred the second period and none at all the last period.

The movement of mineral elements from the plant tissues is shown in the following tabulation (table VII). The direction of most of this transfer is into the grain, with the exception of potassium where there is an actual loss from the plant. The loss of elements from the husks was greatest, amounting to more than half their total content.

TABLE VII

MOVEMENT OF MINERAL ELEMENTS FROM TISSUES

	Nitrogen		Phosphorus		Potassium	
	Max. acc.	Loss	Max. acc.	Loss	Max. acc.	Loss
	gm.	%	*gm.*	%	*gm.*	%
Leaf	1.80	40	0.209	33	1.36	26
Stem	1.61	45	0.334	26	2.68	40
Husk	0.81	69	0.176	66	0.86	51

A study of the accumulation and movement of each element separately was made to show the balance due to the movement from one tissue to another or to account for the total of each element in the plant. The date on which the plant showed the highest accumulation was taken as the starting point and the last sampling date as the other point. The data follow (table VIII):

TABLE VIII

BALANCE OF ACCUMULATION OF NITROGEN

Date	Amount of nitrogen in tissue					
	Grain	Leaf	Stem	Husk	Cob	Whole plant
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
August 28	2.13	1.67	1.42	0.44	0.38	6.04
September 18	3.45	1.18	0.90	0.30	0.29	6.12
Gain or loss	+1.32	−0.49	−0.52	−0.14	−0.09	+0.08

The grain gained 1.32 grams of nitrogen during this period. The whole plant gained 0.08 grams. The loss from all the other tissue equalled 1.24 grams, slightly less than that gained by the grain. Shattering of leaf tissue probably accounts for the difference. These data show clearly that the nitrogen from the other tissues of the plant pass into the grain as it forms and matures. The period for studying the balance due to phosphorus movement was taken at the same time as the nitrogen, although phosphorus did not stop moving into the plant until the end of the sampling period (table IX).

TABLE IX

BALANCE OF ACCUMULATION OF PHOSPHORUS

DATE	AMOUNT OF PHOSPHORUS IN TISSUE					
	GRAIN	LEAF	STEM	HUSK	COB	WHOLE PLANT
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
August 28	0.365	0.194	0.308	0.115	0.088	1.070
September 18	0.793	0.145	0.218	0.059	0.067	1.282
Gain or loss	+0.428	−0.049	−0.090	−0.056	−0.021	+0.212

The data (table IX) show that the grain more than doubled in phosphorus content during the period. The total plant shows a marked increase but it is not equal to the amount accumulated in the grain. Leaf, stem, husks, and cob showed a decrease. The sum of the losses from the leaves, stems, husks, and cob, and the increase in the total plant account for all the phosphorus moving into the grain. It appears that a movement of phosphorus occurs from the other tissues of the plant into the grain as long as it is forming, and that there was a continued absorption of phosphorus from the soil during the same period. A similar study was made on the balance of potassium in the plant, but the period began on August 25 when the plant showed its maximum accumulation. These data are shown in the following tabulation (table X).

TABLE X

BALANCE OF ACCUMULATION OF POTASSIUM

DATE	AMOUNT OF POTASSIUM IN TISSUE					
	GRAIN	LEAF	STEM	HUSK	COB	WHOLE PLANT
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
August 25	0.55	1.17	2.36	0.51	0.24	4.83
September 18	0.90	1.06	1.63	0.42	0.17	4.18
Gain or loss	+0.35	−0.11	−0.73	−0.09	−0.07	−0.65

They differ materially from those data for the other two elements. The grain gained some potassium. The cob, husks, and leaves showed a slight loss. However, the loss from the stems is much greater than the experimental error. This behavior of potassium is rather striking and cannot be entirely accounted for at this time. A probable explanation is that the potassium was partly washed from the leaves and stems back into the soil or simply moved back into the soil through the root system. These results demonstrate that the various mineral elements are absorbed from the soil, move throughout the plant or into different tissues, or are lost from the plant independently of one another.

Conclusions

1. Very extensive experiments are necessary to study the seasonal accumulation and movement of mineral elements in corn.

2. Moving average values of the results adequately show the seasonal trends and movements from one tissue to another. Absolute values were too erratic to be studied satisfactorily.

3. The three mineral elements reported here entered the corn plant and moved from tissue to tissue independently of one another.

4. Nitrogen accumulation in the corn plant reached a maximum at silking time and ceased about four weeks later in the season studied. Nitrogen continued to move into the grain from other tissues until maturity.

5. Phosphorus accumulation did not cease until about maturity. This element also moved into the grain from other tissues.

6. Potassium accumulation reached a maximum about three weeks after silking. There was an actual loss of potassium after that time, largely from the leaves and stems of the plant. No marked accumulation of potassium occurred in the grain.

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NOTE ON FLOWER FORMATION IN THE PINEAPPLE INDUCED BY LOW NIGHT TEMPERATURES[1]

J. VAN OVERBEEK AND HÉCTOR J. CRUZADO

(WITH TWO FIGURES)

Received April 13, 1948

In Puerto Rico pineapples flower during the winter season. It is not known, however, which specific external factors cause this change from the vegetative to the floral apex. Outside of seasonal fluctuations in precipitation, short day and low temperature conditions appear as the only alternatives.

Experiments in which the length of day was varied indicated that the Cabezona variety may respond weakly to changes in the length of day. Long day conditions during the winter tended to keep plants of this variety in the vegetative state (5), while short day conditions during the summer caused a small percentage of Cabezona plants to produce flowers. No responses to photoperiodic treatment were found in the Red Spanish variety. Since this variety flowers much more abundantly than the Cabezona during the winter season it is obvious that photoperiodism is not an important factor in the natural flowering of the pineapple plant. For this reason experiments were begun in which plants of the Red Spanish variety were subjected to night temperatures which approached those of Puerto Rican winters in our locality, an average minimum night temperature of 60–62° F. This temperature is about 10° F. below that of the average minimum summer temperature.

Thrifty young plants of the Red Spanish variety were transplanted from the field into two specially constructed boxes (fig. 1). The boxes were so arranged that by placing 300 lbs. of ice in one of them a minimum night temperature could be obtained which was 10° F. below that of the other box, which had a temperature comparable to that of the outside atmosphere. Thermograph records showed that the major drop in temperature occurs before midnight. After this time the temperature drops slowly until about sunrise. With these facts in mind, ice was placed in the low temperature box at midnight. This box as well as the control box (without ice) were then closed by the covers which were left in place until 10 a.m. the next morning. In order to insure an even distribution of air temperature electric fans operated inside the boxes.

The pineapples were planted in the boxes (about 10 plants per box) on August 8, 1946. On August 19th the experiments with cold night temperatures were begun and continued until September 20th. On October 11th the plants were taken out of the boxes and their growing point examined by cutting them in half longitudinally (fig. 2). In the box with low night

[1] Work carried out at the Institute of Tropical Agriculture of Puerto Rico.

FIG. 1. Pineapple plants growing in a box in which the effect of lowering of the night temperature on flower formation was studied. The photograph shows the control box. The box in which the temperature was lowered during the night had a trough in the center in which 300 lbs. of ice was placed each night. Electric fans provide air circulation.

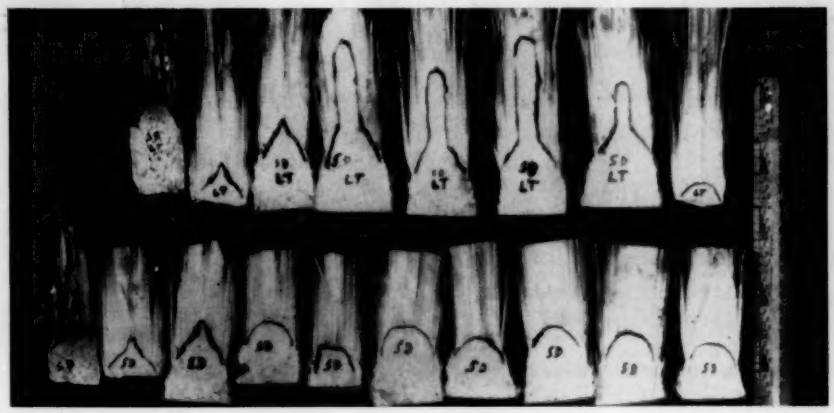

FIG. 2. Longitudinal sections of the upper part of the axis of pineapple plants. Upper row: subjected to a minimum night temperature of about 62° F. The majority of the plants have the young inflorescence and peduncle clearly visible (traced with India ink on the plant material). Lower row: control plants from a box in which the temperature corresponded to that of late summer (minimum night temp. about 72° $F_.$). The dome-shaped outline of the vegetative growing point is clearly visible.

temperature 88% of the plants had formed flowers, while in the control box only 28% of the plants flowered. Comparable plants, planted outside of the boxes, had flowered 30%. The experiment provides direct evidence that flower formation of the pineapple under natural conditions is due to a drop in night temperature during the winter season.

Discussion

Field observations in Hawaii (**1**), together with the direct experimentation reported here seem to point to the conclusion that the drop in night temperature during the winter season is a major *external* factor causing pineapple plants to flower under natural conditions. Laboratory investigations have also accumulated considerable evidence for the hypothesis that an increase in the free auxin level is a major *internal* factor in flower formation in the pineapple plant (**6, 7**). How can the two viewpoints be integrated?

SIDERIS *et al.* (**3**) have shown that the tissues of the pineapple plant undergo diurnal changes in acidity. Malic and citric acids are increased at night, but decreased at day. PUCHER *et al.* (**2**) demonstrated that in Bryophyllum in the dark a drop in temperature resulted in a substantial increase in organic acids. Again malic and citric acids were involved. It has also been reported by THIMANN and his associates (**4**) that, in the presence of malic acid and some other physiologically related acids, auxins increased the rate of respiration in coleoptile sections. Later studies have made it appear likely that auxin is linked with the energy-yielding system of the Krebs cycle. These reports make it possible to visualize a link between lowering of the night temperature, increased auxin level (or activity), and flower formation. This link may be found in the organic acid metabolism.

Summary

Unseasonal flower formation was induced in pineapples of the Red Spanish variety by lowering the minimum night temperature during late summer (about 72° F.) to that approaching minimal winter temperatures (about 62° F.). The idea is presented that the path by which low temperatures cause flower formation in the pineapple is via the organic acid and auxin metabolisms.

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POTASSIUM DEFICIENCY AND EXCESS IN GUAYULE
I. GROWTH RESPONSES AND MINERAL CONTENT[1]

BRUCE J. COOIL

(WITH TWO FIGURES)

Received June 2, 1947

The extensive literature dealing with the role of potassium in plant metabolism has been reviewed by HOFFER (**20**), by various authors (**47**) in a recent symposium and in several reviews on mineral nutrition of plants (**11, 17, 18, 28, 39, 46**). Very recently SIDERIS and YOUNG (**42, 43, 44**) have dealt with several aspects of the problem. HOAGLAND (**19**) has pointed out that at one time or another a role in almost every important physiological process in the plant has been ascribed to potassium. Yet, the mechanism of its role in any specific process has not been elucidated.

In several respects the guayule plant, *Parthenium argentatum* A. Gray, differs from most of the plants which have been subjected to detailed nutritional study. For example, incidence of the reproductive phase in guayule is not attended by cessation of vegetative growth nor by curtailed nutrient absorption. This behavior is contrasted with that of many annual plants, for which a period of rapid growth and mineral absorption is terminated by the reproductive phase. NIGHTINGALE *et al.* (**31**) found that a deficiency of potassium in tomato plants induced premature fruiting. The result was withdrawal of a large portion of the potassium from the vegetative parts to the fruits. RICHARDS and TEMPLEMAN (**40**) found that for the barley plant potassium deficiency resulted in continued tillering and failure of plants to head. The sodium content of the nutrient solutions appears to be a factor associated with the latter result (**39**). No effects analogous to the premature fruiting of tomato plants or to the retarded heading of barley plants are evident for guayule moderately deficient in potassium. The use of guayule plants in a study of potassium nutrition, therefore, has the advantage of permitting comparison between plants which are in different nutritional states, without complications of premature or delayed incidence of the reproductive phase.

When plants are grown in solutions containing insufficient potassium, the absorption of certain other nutrients is increased. Most considerably altered is the tissue content of other cations (**3, 16, 30, 36, 39, 42**), although the contents of anions, particularly phosphorus, may also be effected (**24, 38, 39**). In these circumstances it is difficult to distinguish between effects of potassium deficiency in the tissue and effects arising from accumulation

[1] Contribution from the Guayule Research Project, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture. The cooperation of the U. S. Forest Service is gratefully acknowledged. Clerical assistance was provided by the U. S. Regional Salinity Laboratory, Riverside, Calif.

of other ions. An approach to this problem is the use of more than one series of treatments varying in proportions of cations other than potassium (**3, 11, 38**), or use of series differing in the form in which nitrogen is supplied (**38, 42, 49**). In any case it seems essential to know the tissue contents of each of the principal nutrients under the specific conditions of the experiment.

In this paper are reported growth responses and mineral contents of guayule plants supplied with low, intermediate and high levels of potassium. Two series of treatments differing in supply of calcium and sodium were employed in an attempt to distinguish between effects immediately associated with the potassium content of the tissues and effects resulting from accumulation or deficiency of other nutrients.

Methods

Culture technique

One thousand plants seventeen months of age of strain #593 were selected for uniformity at the Alisal Nursery of the Emergency Rubber Project at Salinas, California, on November 20, 1944. These plants ranged between 6.0 mm. and 9.0 mm. in crown diameter. Tops of all plants were pruned to three inches, thus removing all the leaves. Roots were pruned to four inches. The average dry weight of the pruned material was 3.2 ± 0.06 grams per plant. On November 21, these plants were planted at the rate of four per crock in washed fine gravel in three-gallon glazed crocks provided with drainage. Initial samples were selected at random for control analysis. Tap water was applied until December 23, when approximately 95 per cent of the plants had formed new shoots.

At this time the plants in each crock were thinned to three per crock. One hundred and eighty crocks were selected and arranged in three equal blocks. The sixty crocks within each block constituted six treatment plots of ten crocks each in random arrangement. The compositions of the nutrient solutions supplied are shown in table I. These solutions were prepared from 0.5 M stock solutions of C.P. salts and distilled water. The reactions of the solutions were between pH 5.6 and pH 5.8. The solutions were supplied manually at the rate of 200 ml. twice daily from December 23 until January 25. At this time the rate was increased to three daily applications of 250 ml. each.

The potassium concentrations were selected on the basis of a preliminary experiment which included treatments at five concentrations distributed over a wide range. Treatments K_1 and NaK_1 were expected to result in potassium deficiency, K_2 and NaK_2 to result in optimal growth, K_3 and NaK_3 to provide excessive potassium. The treatments comprised two series, series 1 being characterized by a moderate calcium concentration. In series 2, sodium was partially substituted for calcium to result in the same total ion-equivalent concentration as in series 1 for any given potassium level. Magnesium and phosphorus were supplied at uniform levels

throughout all treatments. Sulphate was the form in which variables of potassium were supplied.

The plants were removed in two harvests. The first harvest was on February 6 and 7, and the second on March 15, 16, and 17. For simplicity these harvests hereafter are referred to as those of February 6 and March 16. Plants from given blocks, representing all treatments, were removed on each date. All plants were harvested between 8:00 a.m. and 11:00 a.m. except in the case of the second block at the first harvest. This block was harvested between 1:00 p.m. and 3:00 p.m. on February 6. At each harvest the plants from one half the crocks in each plot were removed, (i.e., 45 plants per treatment) so the randomized arrangement was maintained. The greenhouse temperature was above 60° F. at all times. The average daily minimum temperature for the period was 68° F., average daily maximum temperature 80° F.

TABLE I

COMPOSITION OF THE NUTRIENT SOLUTIONS*

SERIES	TREATMENT DESIGNATION	EQUIVALENTS PER LITER				
		$Ca(NO_3)_2$	$NaNO_3$	K_2SO_4	$MgSO_4$	KH_2PO_4
1	K_1	0.0100	.....	.....	0.0020	0.0005
	K_2	0.0100	.....	0.0025	0.0020	0.0005
	K_3	0.0100	.....	0.0115	0.0020	0.0005
2	NaK_1	0.0040	0.0060	.....	0.0020	0.0005
	NaK_2	0.0040	0.0060	0.0025	0.0020	0.0005
	NaK_3	0.0040	0.0060	0.0115	0.0020	0.0005

* In addition each solution contained: 1.0 p.p.m. Fe as ferric citrate, 0.5 p.p.m. B as H_3BO_3, 0.5 p.p.m. Mn as $MnCl_2$, 0.05 p.p.m. Zn as $ZnSO_4$, 0.02 p.p.m. Cu as $CuSO_4$, 0.01 p.p.m. Mo as H_2MoO_4.

GROWTH MEASUREMENTS AND TISSUE PREPARATION

Several measurements of growth were employed. In addition to determination of total fresh weights and dry weights, it was desired to have indexes of relative activity of stem tip and cambial meristems. For this purpose the longest shoot on each plant was selected for measurement. The "length of shoot" was taken as the distance from the point this shoot branched from the original stock to the highest leaf apex when flattened vertically. Trials showed that this measurement could be reproduced within about 1 cm. The "diameter of shoot" measurement was the average of two caliper readings taken at right angles to each other 1 cm. above the point of branching of the shoot from the original stock. Because the difference in these measurements resulting from nutrient treatments would not be large in any case, as large a number of observations as possible was desired. To this end measurements of length and diameter were obtained from each plant. With the same considerations with regard to number of observations, the fresh weight of the three plants from each crock was determined.

The procedure for obtaining weights and measurements and preparing the plant parts for analysis was as follows: The roots were washed free of gravel and rinsed in distilled water. After draining for a few seconds, remaining free water was blotted from them with toweling. Fresh weights were determined, and measurements of length and diameter of shoots made. The plants were then separated into the following parts: (1) mature leaves, all leaves below the eighth leaf on each branch; (2) expanding leaves, the eight youngest leaves on each branch. This actually included all expanding leaves and one or two fully expanded leaves; (3) young stem, all the stem growth resulting from the shoots produced since transplanting; (4) old stems; and (5) roots from the original stock separated at the crown; (6) flowers and fruit including the floral stalk; and (7) dead leaves, including all that had fallen during the treatment interval. After these separations the corresponding parts from all plants of the same plot were combined. Fresh weights were determined. Woody portions were chopped into small pieces. Samples were thoroughly mixed and portions dried to constant weight of 65° C. in a forced draft oven. The total dry weight of each plant part was determined. Composite samples were made by thoroughly mixing portions of dried samples from all plant parts other than the leaves. In these composite samples, each plant part was represented in the same proportion as originally present in the whole plants.

At the March 16 harvest 100-gram portions of the fresh leaf samples were frozen for sap expression. Details of the methods employed in freezing and thawing as well as the results obtained from the frozen samples are reported in another paper of this series (**5**).

Methods of analysis

Total calcium and magnesium contents of the leaves were determined by analysis of sap and press cakes from frozen samples (**5**). For the analysis of other constituents of the leaves and for all constituents of the composite samples, dried material was employed. Five-hundred-milligram portions of dried, finely ground material from the composite or leaf samples were weighed into porcelain capsules, moistened with sulphuric acid (1–10), dried at 105° C. and ashed at 600° C. for 16 hours. The ash was dissolved in dilute HCl, taken to dryness to dehydrate any silica, and redissolved in HCl. Calcium, magnesium, and sodium in the ash were determined by the official methods (**1**). Potassium was determined by the method of Hibbard and Stout (**14**). Sulphur and phosphorus were determined in ash prepared by the magnesium nitrate method (**1**). Sulphur was determined by the official method (**1**). A separate aliquot of the ash solution was used for determination of phosphorus by the method of Fiske and Subbarow (**8**). Total nitrogen including nitrate was determined by the modification of the procedure of Pepkowitz and Shive (**32**) described by Kelley *et al.* (**25**).

Samples from each of the eighteen plots (i.e., three replicates of each treatment) were analyzed separately. Analysis of variance (**45**) was

applied to the analytical data as well as to the growth data. Coefficients of correlation and indexes of correlation were determined as described by EZEKIEL (7).

Results

LEAF SYMPTOMS

At the time of the first harvest (February 6) symptoms of potassium deficiency in treatments K_1 and NaK_1 were beginning to appear. Yellow-green circular areas on the older leaves could be observed by transmitted light. No difference in degree of these symptoms could be observed between these two treatments. On February 24, premature death of the oldest leaves of plants of these two treatments could be noted. Drying of these leaves frequently progressed basipetally from the apex. In other cases, the original chlorotic spots became brown and dried first. At this time, it was noted that the entire foliage of plants receiving the K_3 and NaK_3 treatments was more yellow-green in color than the foliage of plants receiving the other treatments. These symptoms were still evident at the time of final harvest (March 16).

Throughout the experiment a few extra crocks of plants had been supplied with the same six treatments as those of the main experiment. These plants were maintained by continued application of these solutions for two additional months, so further development of the symptoms could be observed. The plants receiving the K_1 and NaK_1 treatments eventually lost nearly all their mature leaves, but continued to grow, continually forming young leaves. The foliage of plants receiving the K_3 and NaK_3 treatments became more yellow, and the older leaves showed a purple coloring suggestive of phosphorus deficiency.

GROWTH

As measures of total growth the total fresh weight per plant and total dry weight per plant are used. These results as well as those for percentage dry matter for the whole plants are shown in table II. At the time of the first harvest (February 6) the K_3 treatment had resulted in greater fresh weight than any other treatment, whereas, NaK_3 had resulted in the least amount of fresh weight and dry weight. These comparisons are statistically significant for fresh weights. Among the other four treatments, differences in fresh weight or dry weight were not significant at this time. Percentage dry matter was significantly lower in K_3 than in K_1 or NaK_3.

On March 16, total fresh weight was less in series 2 than in series 1 at any given level of potassium supply. Within each series total fresh weight and dry weight were significantly reduced by the low potassium treatment (i.e., K_1 was less than K_2 and NaK_1 less than NaK_2). High potassium treatment resulted in reduction in growth only in series 2. Percentage dry matter for the whole plants did not differ significantly among treatments.

TABLE II

TOTAL FRESH WEIGHT, DRY WEIGHT AND PERCENTAGE DRY WEIGHT

TREATMENT	TOTAL FRESH WEIGHT GRAMS PER PLANT		TOTAL DRY WEIGHT GRAMS PER PLANT		PERCENTAGE DRY MATTER	
	FEB. 6	MAR. 16	FEB. 6	MAR. 16	FEB. 6	MAR. 16
K_1	28.8	68.6	5.64	15.2	19.6	22.2
K_2	30.4	80.0	5.64	18.8	18.6	23.5
K_3	34.2	82.2	6.09	18.2	17.8	22.2
NaK_1	28.1	62.6	5.34	14.3	19.0	22.9
NaK_2	28.2	70.6	5.33	16.0	18.9	22.7
NaK_3	23.7	65.9	4.69	14.0	19.8	21.2
Difference required for significance						
0.05	3.17	5.96	0.558	1.45	1.40	n.s.
0.01	4.22	7.93	0.793	2.06	1.98	.....

In table III are shown the dry weights of the several parts into which the plants were separated. There are a few points which could not have been seen from the weights of the whole plants. On March 16, the dry weights of expanding leaves from treatments K_1 and K_2 were nearly identical; whereas, the weights of mature leaves in common with the weights of other live portions were less in K_1 than in K_2. The lower weights of mature leaves in K_1 may be partially accounted for by the greater weight of dead leaves in this treatment. In series 2, analogous differences were not found,

TABLE III

DRY WEIGHT OF PLANT PARTS

PLANT PART	TREATMENTS					
	K_1	K_2	K_3	NaK_1	NaK_2	NaK_3
	FEBRUARY 6					
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Expanding leaves	0.79	0.67	0.77	0.70	0.67	0.45
Mature leaves	1.24	1.35	1.45	1.21	1.24	1.14
Flowers plus fruits	0.30	0.29	0.27	0.30	0.30	0.18
Young stems	0.55	0.57	0.57	0.54	0.50	0.35
Old stems	1.52	1.51	1.65	1.44	1.44	1.41
Roots	1.24	1.25	1.38	1.15	1.18	1.16
Total	5.64	5.64	6.09	5.34	5.33	4.69
Dead leaves	0.06	0.08	0.28	0.11	0.25	0.26
	MARCH 16					
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Expanding leaves	3.87	3.92	3.70	2.94	3.56	2.89
Mature leaves	2.49	3.69	4.12	2.98	3.22	2.86
Flowers plus fruit	1.43	1.79	1.59	1.23	1.39	1.07
Young stems	3.41	4.76	3.93	3.17	3.34	2.90
Old stems	1.87	2.16	2.25	2.03	2.13	2.11
Roots	2.14	2.49	2.66	1.99	2.39	2.16
Total	15.21	18.81	18.25	14.34	16.03	13.99
Dead leaves	1.93	1.47	1.38	1.20	1.28	1.62

rather the weight of both young and old leaves were lower in NaK_1 than in NaK_2. In comparing treatment K_2 with K_3, it is notable that on March 16 the weight of young stems was much higher in K_2. The weights of flowers plus fruits and of expanding leaves were slightly higher in K_2. However K_3 had the greater weight of mature leaves and slightly greater weights of old stem and roots. In series 2, NaK_3 had a lower weight of each live part and a greater weight of dead leaves relative to NaK_2.

Measurements of diameter and length of the longest shoot are shown in table IV. On February 6, shoot diameters in K_3 were significantly greater than in any other treatment except NaK_3. The only other comparison representing a significant difference is that between K_1 and NaK_3. On March 16, shoot diameters were significantly smaller in K_1 than in K_2, and significantly smaller in K_1 or NaK_1 than in K_3 or NaK_3. It is noteworthy that although certain individual comparisons did not show significant differences, a general trend of increasing diameter growth was asso-

TABLE IV

DIAMETER AND LENGTH OF THE LONGEST SHOOT

	Treatments						Differences required for significance	
	K_1	K_2	K_3	NaK_1	NaK_2	NaK_3	0.05	0.01
	February 6							
Diameter (mm.)	3.08	3.18	3.49	3.16	3.24	3.35	0.24	0.31
Length (cm.)	24.1	23.8	24.1	24.5	22.2	19.4	1.38	1.82
	March 16							
Diameter (mm.)	4.73	5.12	5.36	4.83	5.03	5.36	0.37	0.48
Length (cm.)	30.6	30.1	27.1	30.3	27.6	25.7	1.21	1.60

ciated with increasing potassium supply in each series at each harvest. On February 6, lengths of shoots were less in NaK_2 and NaK_3 than in other treatments, and less in NaK_3 than in NaK_2. Among the other four treatments differences were not significant. At the final harvest, K_3 may be classed with NaK_2 and NaK_3 as less than the other three treatments. At this harvest as at the first, lengths of shoots were less in NaK_3 than in any other treatment.

It is evident from the data presented that the growth response to the treatments employed was rather complex. Thus, although the low potassium treatment had reduced the fresh weights and dry weights of plants in each series at final harvest, the high potassium treatment resulted in reduction in growth only in series 2. A general difference in growth response to the two series of nutrient treatments was indicated. Variations in diameter and length of shoot were not correlated, and indicated alteration in the form of the plants by the various treatments. Before interpretation of these results is attempted, however, the mineral contents of the plants will be considered.

Mineral content

The percentage composition of leaf tissues and of composite samples at the February 6 and March 16 harvests are recorded in tables V and VI respectively. In interpreting these results it is, of course, evident that a higher concentration in leaf tissues than in the composite samples does not necessarily mean that the concentration in the leaves is higher than in any other plant part, but rather the composition of the composite sample repre-

TABLE V

CONCENTRATION OF ELEMENTS IN LEAF TISSUES AND IN COMPOSITE SAMPLES AND PROPORTIONS OF TOTAL QUANTITIES PRESENT IN THE LEAVES ON FEBRUARY 6

Treatment	Tissue	Percentage of the dry weight						
		K	Na	Ca	Mg	N	P	S
K_1	Expanding leaves	2.66	0.26	4.63	0.75	5.98	0.471	1.22
	Mature leaves	1.84	0.28	6.27	1.40	4.20	0.415	1.52
	Composite*	1.76	0.20	0.60	0.15	1.27	0.412	0.23
K_2	Expanding leaves	5.63	0.11	3.71	0.78	5.40	0.446	1.33
	Mature leaves	4.50	0.11	5.05	1.05	4.38	0.359	1.49
	Composite	2.85	0.13	0.58	0.10	1.33	0.440	0.23
K_3	Expanding leaves	7.62	0.06	3.07	0.69	4.80	0.440	1.17
	Mature leaves	7.66	0.05	3.65	0.85	4.77	0.325	1.62
	Composite	4.46	0.08	0.52	0.08	1.32	0.418	0.30
NaK_1	Expanding leaves	4.03	0.70	3.45	1.19	5.49	0.471	1.17
	Mature leaves	3.48	0.59	4.29	1.75	4.31	0.359	1.38
	Composite	1.60	0.74	0.50	0.15	1.34	0.434	0.24
NaK_2	Expanding leaves	5.36	0.44	3.09	1.00	5.17	0.443	1.15
	Mature leaves	4.89	0.44	3.23	1.35	4.37	0.304	1.46
	Composite	2.78	0.48	0.50	0.11	1.25	0.428	0.23
NaK_3	Expanding leaves	7.27	0.23	2.14	0.83	4.58	0.459	1.23
	Mature leaves	6.72	0.23	2.42	1.05	4.20	0.297	1.65
	Composite	3.99	0.26	0.44	0.09	1.16	0.462	0.22
		Quantity in the leaves Percentage of quantity present in the whole plants						
Average of all treatments		48.4	33.4	80.0	84.3	52.8	33.1	77.7

* Composite samples of all tissues other than leaves. Each tissue is represented in the same proportion as found at harvest.

sents an average composition of all other parts. With the exceptions of sodium and phosphorus the concentrations were generally higher in the leaves than in the composite samples. With respect to potassium, a notable exception was the K_1 treatment on March 16. Also shown in tables V and VI are the amounts of nutrients in the leaves expressed as percentage of the amounts present in the whole plants. These values are, of course, based on the proportion of the whole plant represented by leaf tissue, as well as the concentrations in the tissues. These figures illustrate the relatively very high proportions of calcium, magnesium, and sulphur present in the leaves.

The low proportion of sodium in the leaves on March 16 is of interest. Although the proportion was somewhat higher in NaK_3 than in other treatments, the distribution of this element was quite different from that of potassium. These results do not show in what plant part the sodium was concentrated, but they recall the higher percentage of sodium in trunks and roots than in other parts of the orange trees studied by REED and HAAS (**36**), the higher concentrations in roots than in tops of a number of plants

TABLE VI

CONCENTRATION OF ELEMENTS IN LEAF TISSUES AND IN COMPOSITE SAMPLES AND PROPORTIONS OF TOTAL QUANTITIES PRESENT IN THE LEAVES ON MARCH 16

TREATMENT	TISSUE	PERCENTAGE OF THE DRY WEIGHT						
		K	Na	Ca	Mg	N	P	S
K_1	Expanding leaves	1.49	0.02	5.79	1.02	5.15	0.307	1.92
	Mature leaves	1.41	0.05	7.19	1.17	4.12	0.276	2.12
	Composite*	1.60	0.23	0.92	0.21	1.89	0.390	0.28
K_2	Expanding leaves	4.14	0.02	4.61	0.84	5.04	0.270	1.84
	Mature leaves	5.40	0.02	5.79	0.89	4.05	0.220	2.40
	Composite	2.70	0.11	0.80	0.13	1.89	0.412	0.27
K_3	Expanding leaves	8.99	0.05	2.44	0.54	4.45	0.254	2.29
	Mature leaves	10.01	0.00	3.25	0.51	3.68	0.183	2.82
	Composite	3.91	0.07	0.60	0.13	1.74	0.452	0.44
NaK_1	Expanding leaves	2.70	0.23	3.75	1.37	5.14	0.307	1.90
	Mature leaves	2.54	0.37	5.29	1.69	4.06	0.297	2.40
	Composite	1.25	0.87	0.52	0.19	1.75	0.434	0.34
NaK_2	Expanding leaves	4.73	0.30	3.47	1.02	4.86	0.285	1.99
	Mature leaves	6.33	0.39	4.27	1.20	3.96	0.288	2.61
	Composite	2.35	0.53	0.62	0.15	1.76	0.459	0.39
NaK_3	Expanding leaves	8.89	0.20	2.00	0.62	4.27	0.248	2.13
	Mature leaves	10.67	0.22	2.32	0.66	3.78	0.220	2.83
	Composite	3.83	0.19	0.54	0.13	1.72	0.489	0.38
	QUANTITY IN THE LEAVES PERCENTAGE OF QUANTITY PRESENT IN THE WHOLE PLANTS							
Average of all treatments		57.4	20.3	81.9	80.4	63.9	29.9	82.3

* Composite samples of all tissues other than leaves. Each tissue is represented in the same proportion as found at harvest.

studied by COLLANDER (**4**), and similar results obtained by HAYWARD *et al.* (**13**) for peach trees.

To evaluate the absorption of individual and total cations and anions under the conditions of this experiment, the total mineral contents of the plants have been calculated in terms of milliequivalents. Each element is expressed in the ionic form supplied in the nutrient solutions (e.g., total nitrogen as NO_3^-, total phosphorus as $H_2PO_4^-$ and total sulphur as $SO_4^=$). These are the forms in which these elements are assumed to have been absorbed, notwithstanding subsequent assimilation within the plants. In table VII cation and anion contents, before and after applying the nutrient treatments, are expressed as milliequivalents per plant. From the growth

data it is evident (table II) that among the treatments K_1, K_2, NaK_1, and NaK_2 there were no significant differences in total dry weights or fresh weights on February 6. Direct comparisons of absorption of nutrients by plants of these treatments are possible. For comparisons involving the other two treatments at this date and all treatments on March 16, differences in growth must be considered.

Differences in potassium concentration in the nutrient solutions resulted in large differences in potassium absorption. Only relatively small differences in potassium contents were evident between K_1 and NaK_1, or between

TABLE VII

MINERAL CONTENT OF WHOLE PLANT

TREATMENT	MILLIEQUIVALENTS PER PLANT						
	K	Na	Ca	Mg	N	S	P
	INITIAL ANALYSIS						
	1.36	0.035	0.50	0.18	1.12	0.38	0.31
	FEBRUARY 6						
K_1	2.7	0.55	6.8	2.4	10.4	2.3	0.76
K_2	5.1	0.30	5.6	1.9	10.3	2.3	0.79
K_3	8.8	0.18	4.8	1.7	10.7	2.8	0.79
NaK_1	3.2	1.62	4.6	2.9	9.8	2.2	0.73
NaK_2	5.2	1.09	3.9	2.2	9.4	2.3	0.68
NaK_3	5.9	0.53	2.5	1.6	7.5	2.4	0.64
DRFS* 0.05	0.89	0.24	0.56	0.26	1.13	n.s.	0.084
0.01	1.27	0.38	0.80	0.37	1.61		0.119
	MARCH 16						
K_1	6.0	0.95	24.2	7.1	33.4	9.5	1.8
K_2	17.0	0.55	24.1	6.6	38.4	12.0	2.1
K_3	28.4	0.33	14.3	4.5	35.5	15.4	2.1
NaK_1	6.7	4.0	15.3	8.8	29.9	9.7	1.8
NaK_2	15.1	3.1	15.8	7.3	33.1	11.9	1.9
NaK_3	22.3	1.2	8.4	3.9	26.6	11.1	1.7
DRFS* 0.05	1.96	0.07	1.98	0.77	3.10	1.16	0.28
0.01	2.78	0.12	2.81	1.09	4.41	1.64	0.39

* Difference required for significance.

K_2 and NaK_2. At the high potassium level the considerable difference in size of the plants precludes direct comparison.

Calcium absorption was significantly decreased with each increment of increase in potassium concentration in each series during the period preceding February 6. The difference in calcium concentrations of the nutrient solutions of series 1 and series 2 resulted in differences in calcium contents of the plants at each level of potassium supply. The magnesium concentration was identical in all solutions. Therefore, all differences in absorption of this nutrient were affected by variations of concentrations of the other nutrients. Absorption of magnesium as that of calcium decreased

with each increment of increase in potassium supply. At low and intermediate levels of potassium supply, magnesium absorption was higher in series 2 than in series 1.

Absorption of sodium decreased with each increment of increase in potassium concentration. Although relatively high concentrations of sodium were supplied (table I) absorption of this element was very low compared to that of the other cations. This is particularly evident when it is considered that the solution concentration of sodium in series 2 was higher

TABLE VIII

MINERAL CONTENT OF WHOLE PLANTS

TREATMENT	MILLIEQUIVALENTS PER 100 GRAMS OF DRY TISSUE								
	K	Na	Ca	Mg	TOTAL CATIONS	N*	S*	P*	TOTAL ANIONS
	INITIAL ANALYSIS								
	44	1.1	16	5.7	66.8	37.4	10.0	12.1	59.4
	FEBRUARY 6								
K_1	48	9.8	120	42	221	185	41	13.5	239
K_2	91	5.3	99	33	228	183	41	13.7	237
K_3	145	3.0	79	28	255	177	45	13.0	235
NaK_1	60	30.0	87	54	231	183	41	13.6	238
NaK_2	97	20.0	73	42	232	176	43	12.8	232
NaK_3	126	11.0	54	34	226	159	51	13.7	224
DRFS† 0.05	21	3.1‡	9.8	4.1	n.s.	8.4	5.5	n.s.	9.6
0.01	29	4.8	13.9	5.7	.....	11.9	7.8	.....	n.s.
	MARCH 16								
K_1	39	6	159	47	251	220	62	11.6	294
K_2	90	3	128	35	256	205	64	11.2	280
K_3	155	2	78	25	260	195	85	11.3	291
NaK_1	47	28	108	61	244	209	68	12.1	289
NaK_2	94	20	99	45	258	206	74	11.8	292
NaK_3	160	9	60	28	257	186	79	12.4	277
DRFS† 0.05	6.8	3.9‡	8.5	0.9	n.s.	14.8	5.8	n.s.	n.s.
0.01	9.7	6.5	12.0	1.2	.....	21.1	8.3	.....	.....

* Anions are assumed to have been absorbed in the ionic forms supplied in the nutrient solution, i.e., NO_3^-, $SO_4^=$, and $H_2PO_4^-$.

† Difference required for significance.

‡ Variance analyses for sodium are based on treatments of series 2 only.

than that of magnesium in any treatment, of potassium at low and intermediate levels, and of calcium in series 2. A small but significant absorption resulted in series 1 where sodium was not deliberately supplied.

The absorption of nitrogen, sulphur and phosphorus was affected relatively little by differences in treatments employed. The bulk of the anion absorption was accounted for as nitrate. Absorption of this ion was slightly lower in series 2. However, individual comparisons are not significant except at the high potassium level. The low phosphorus absorption was related to a low solution concentration of this nutrient. Despite the rela-

tively wide range of sulphate concentration supplied, no significant difference in absorption of this nutrient had resulted at the time of the first harvest.

In the above comparisons mineral contents have been considered in terms of milliequivalents per plant. This is undoubtedly the most direct basis for comparison, but is strictly applicable only where treatments have not resulted in differences in growth. Where such differences have resulted, some information on relative absorption may be obtained by expressing the quantities in terms of concentration. McCALLA and WOODFORD (**30**) utilized this method in their study of effects of limiting one element in wheat. In the present experiment the number of comparisons can be increased to include the K_3 and NaK_3 treatments at the first harvest and all treatments at the second harvest. In table VIII, individual and total cation and anion contents of whole plants are expressed in terms of milliequivalents per 100 grams of dry tissue. It may be seen that differences in potassium concentration within the plant were compensated by reciprocal differences in concentrations of other cations, thus maintaining a nearly constant total cation concentration for a given age of plant. Similarly, decreased calcium concentration in plants from the series 2 treatments were compensated by increases in sodium, magnesium and in most cases, slight increases in potassium concentration.

The total equivalent concentration of anions was nearly constant among the various treatments. Relatively small decreases in nitrogen content of plants receiving the high potassium treatment were in several cases associated with small increases in sulphur content. It is evident, however, that although solution concentrations of sulphate varied considerably, relatively little difference in total absorption of this ion resulted.

Discussion

The difficulty of predicting the effect of a given concentration of an ion in the culture solution upon the amount of that ion found in the plant is well known (**12**, **33**, **37**). For example, PIERRE and BOWER (**33**) list six of the factors which may influence the effect of other cations upon the absorption of potassium: level of K, concentrations of cations in relation to K, kind of cation, presence of other cations, kind of plant, pH of solution. It might be noted that the absorption of each of the cations present in the solution may be influenced by each of these factors.

McCALLA and WOODFORD (**30**) found that in wheat, limiting the supply of one nutrient resulted in an increased uptake of another nutrient absorbed as an ion of the same sign, or in a decreased total uptake of the nutrients as ions of the opposite sign. In the present experiment the absorption of individual and total anions was affected very little by the treatments. HOAGLAND (**16**) reported that in numerous water culture experiments decreasing percentages of potassium in the plant tissues were accompanied by increasing percentages of calcium and magnesium. Occasionally there was

found a nearly exact substitution of bases, but generally the total equivalents of bases absorbed by the plant was greater under high potassium conditions than under low. In several other cases it has been observed that where conditions of growth were similar, except for nutrient composition of the substrate, "ion substitution," or "mutual replacement" may take place to the extent that the total equivalent concentration of cations within the plant remains nearly constant, notwithstanding extreme variations in concentrations of individual cations (**2, 21, 22**). In the present experiment, the high potassium plants had slightly higher total cation concentrations in several cases, but these differences were not statistically significant (table VIII). In any case these differences were not great and the total cation content must be considered as nearly constant among the various treatments at either time of harvest. Under such conditions it is evident that an alteration in the absorption of any one of the cations results in an altered absorption of one or more of the other cations. Evidently the frequently observed accumulation of calcium or of calcium and magnesium in potassium deficient plants (**3, 16, 30, 36, 39, 42**) as well as the accumulation of potassium or of potassium and magnesium in calcium deficient plants (**30, 35, 36**) cannot be avoided, but only altered.

From these ionic substitution relationships arise the difficulty of distinguishing responses directly associated with deficiency of one cation from those which may be attributed to accumulation of other cations (**9, 10, 15, 39**). The tissue contents in this experiment with guayule present one advantage in this respect. There are three pairs of treatments comparable with respect to concentrations of potassium in the tissues, but differing in concentrations of calcium and magnesium. It is evident that a response which could be plotted as a continuous relationship to potassium content over the whole range of treatments could not be represented as a continuous relationship to calcium, or, for that matter, to magnesium or sodium.

At each harvest date a relationship between potassium content and shoot diameter was found. Correlation coefficients were + 0.616 and + 0.671 for plants harvested February 6 and March 16, respectively. Both coefficients were highly significant. Plots for the March 16 harvest are shown in figure 1. Individual points represent the separate plots. Treatment means are indicated by circles. A relationship between potassium and cambial activity has been indicated by other workers. With the aid of microchemical studies JANSSEN and BARTHOLOMEW (**23**) noted that in tomato plants grown in solutions lacking potassium, this element seems to be transferred to and localized in the meristematic and growing portions of the plant. NIGHTINGALE *et al.* (**31**) observed that when present in the plant in limited amounts, potassium is translocated freely from mature tissues to regions of active cell division. A condition in potassium deficient tomato plants was described where there was insufficient potassium to maintain cambial activity in the older portions of the stem, but enough potassium was present for active development of the meristematic stem tip.

The result was a considerable increase in length of the stems but very little increase in diameter. WALL (**49**) working with tomato plants observed that potassium deficiency had a greater effect on the weights of the plants than it had on the lengths of the tops. That the response in diameter growth to potassium is not confined to stem tissues is pointed out by NIGHTINGALE *et al.* (**31**). Beets deficient in potassium increase mainly in length and little in diameter. In sweet potatoes (**41**) a similar response was observed.

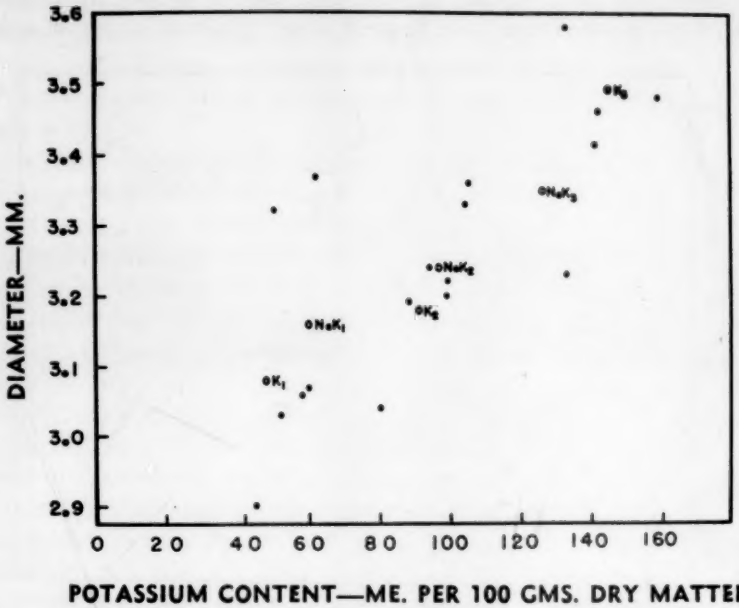

FIG. 1. Relationship between total potassium content and growth in diameter of the shoots for plants harvested March 16. Correlation coefficient = + 0.671. Correlation coefficient required for significance = 0.590 (n = 18, p = 0.01).

The results of the present experiment in contrast with those cited above indicate a response in diameter growth of the shoots over a wide range of potassium supply rather than only in the range where growth is limited by deficiency of this nutrient. In data recently reported by CULLINAN and BATJER (**6**) a similar trend is indicated in the peach tree. With increasing concentrations of potassium supply, the circumference of the trees appears to have increased beyond the range where the total weights of the plants increased.

When an attempt is made to relate the lengths of the shoots to the nutrient composition of the plants, it is noted that variations in calcium content (table VIII) parallel variations in shoot lengths (table IV), particularly in the range of low calcium contents. However, if one were to define the limiting tissue concentration of calcium in terms applicable to

various stages of development of the plant, selection of a plant part of a given physiological age would be necessary. In addition the concentration of soluble calcium is expected to be more closely related to the metabolic processes of the plant than is the total calcium which may include a considerable portion of insoluble, relatively inert salt. The calcium content of the sap expressed from frozen and thawed leaves of plants harvested March 16 has been determined, and from these determinations the "sap-soluble" fraction has been estimated. The details of these results are considered elsewhere (**5**) and only a portion of the results are employed for the immediate consideration. In figure 2 length of shoot is plotted against

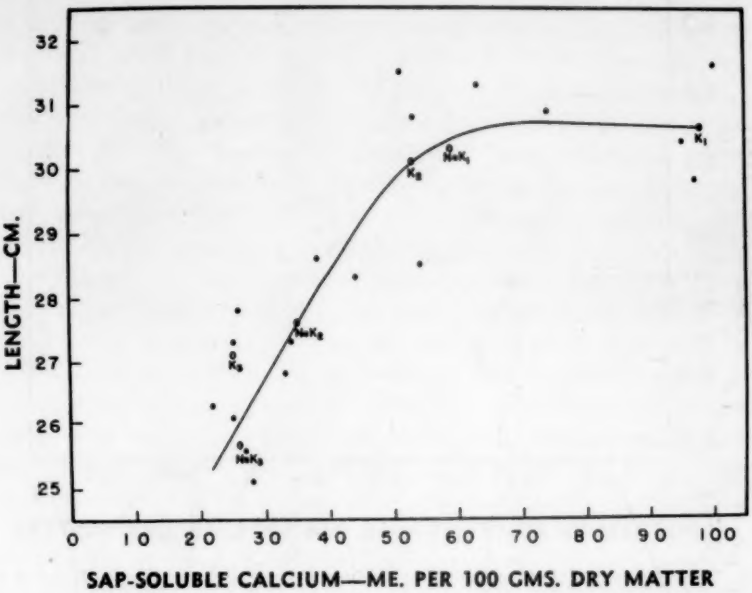

FIG. 2. Curvilinear relationship between sap soluble calcium content of the expanding leaves and growth in length of shoots. Plants harvested March 16. Small dots represent individual plots. Circles denote treatment means. Index of correlation = + 0.893.

the sap soluble calcium content of the expanding leaves. These plots appear to define a limiting relationship. A free hand curve has been fitted to these data as described by EZEKIEL (**7**). The index of correlation is + 0.893. Although here a highly significant relationship is shown, the range where calcium concentration limits growth in length of shoot cannot be defined on this basis with certainty, insofar as the length measurements represent the total growth during the whole period of nutrient treatment, whereas, the calcium contents are those at final harvest. In any case, it may be noted that the relationship between soluble calcium and length of shoot is essentially linear if the K_1 treatment is omitted. The plots may, then, be interpreted as indicating that growth in length of shoot was re-

lated to the soluble calcium concentration in the expanding leaves except where potassium content limited farther increase in length.

The relationship indicated between length of shoot and soluble calcium content of the expanding leaves is not at variance with frequent observations that the terminal meristems of root and stem tips are affected by calcium deficiency. For example HAAS and REED (**12**) found that in the roots of walnut seedlings the terminal meristems were the first tissues affected in solutions lacking calcium, although the terminal portions of the roots were actually higher in calcium content than were segments of the roots further removed from the meristems. GAUCH (**10**) found that the terminal stem meristems of bean plants growing in solutions lacking calcium, ceased growth before the axillary meristems, although the latter too eventually died.

Admittedly significant correlations do not of themselves demonstrate direct cause and effect relationships. In particular it should not be infered that growth in length of the shoots is related only to calcium and growth in diameter only to potassium. In fact REED (**34**) demonstrates that potassium and calcium are both necessary for completion of cell division. It was found that mitotic cell division did not take place without a suitable supply of potassium, although a certain amount of growth by stretching was possible. In the absence of calcium, cells of Spirogyra divided mitotically, but, new cell walls were formed imperfectly, if at all. The inability to form cellulose was also exhibited by the zygote of Spirogyra. Sections of the growing region of the root of corn plants which had grown in solution lacking calcium showed a number of cases in which the transverse septum had not formed after the process of nuclear division was complete.

Without specific reference to the mechanisms involved, aside from the proposition that adequate concentrations of both potassium and calcium are necessary for normal cell division, differential growth responses may be attributed to difference in mobility between potassium and calcium within the plant. As indicated above, potassium is highly mobile and when present in limiting amounts may be readily translocated from more mature tissue to terminal meristems with the result that little or no increase in diameter takes place although growth in length may continue. It is not, then, surprising that an increase in potassium content results in a relatively greater response in diameter growth than in growth in length of the guayule shoots. In contrast to potassium, calcium may be more slowly retranslocated from mature tissue to more terminal portions of the plant. For example, REED and HAAS (**35**) found that where calcium salts were withheld from young orange trees, the trunks and roots were the last to be depleted of calcium. MASON and MASKELL (**29**) presented evidence indicating that in the cotton plant potassium and phosphorus may be re-exported from the leaves via the phloem and transported downwards towards the roots or upwards into the growing bolls, while calcium is re-exported much more slowly, if at all.

In the present experiment it may be noted (tables V and VI) that the calcium content of the expanding leaves was invariably lower than that of the mature leaves, whereas the potassium content of expanding leaves equaled or exceeded that of the mature leaves in the low potassium treatments (K_1 and NaK_1). These observations may be taken as evidence of the different mobilities of these elements in the guayule plant. In this connection it should be noted that the potassium in the leaves is almost entirely soluble whereas a considerable portion of the calcium of the leaves is insoluble (**5**).

Another consideration involves the differential calcium requirements of various plant parts. If the plants of NaK_3 were actually deficient in calcium it is evident that the calcium requirement of the guayule leaves is relatively high, inasmuch as the expanding leaves of these plants had a calcium content of 2.00 per cent of the dry weight on March 16. The average content of parts other than leaves was much lower, as indicated by the composition of the composite samples. Samples of young and old stems from the plants harvested on February 6 were analyzed separately for calcium. Results of these analyses (table IX) show that the stems had lower calcium contents which were not subject to as great variation resulting from nutrient treatments as were those of the leaves (table V).

TABLE IX

CALCIUM CONTENT OF STEM SAMPLES OF PLANTS HARVESTED FEBRUARY 6

TREATMENT	YOUNG STEMS % OF DRY MATTER	OLD STEMS % OF DRY MATTER
K_1	1.00	0.63
K_2	0.81	0.61
K_3	0.76	0.59
NaK_1	0.78	0.54
NaK_2	0.77	0.45
NaK_3	0.67	0.45

The growth response in length of shoots may be explained on the basis that the meristematic tissues of the stem tips and expanding leaves must be supplied from calcium currently absorbed and not supplemented by retranslocation from mature tissues, with the consideration that the expanding leaves have a high requirement for calcium. The stem tissues have a lower calcium requirement, which was satisfied in plants of all treatments to the extent that growth in diameter was not limited by calcium.

Although the relationships between these single nutrients and measurements of length and diameter of the shoots could not be expected to hold where one nutrient becomes severely limiting, and total growth is greatly affected, it is rather striking that they can be shown over as wide a range of tissue concentrations as presented by the plants of this experiment. For example, a comparison of weights of plants from treatments K_3 and NaK_3 (table II) shows a considerable difference in size of these plants at

final harvest, yet the diameters were essentially the same. Growth in NaK_3 appears to have been limited only in elongation of the shoots. On the other hand the limitation of growth in low potassium treatments was not associated with less growth in length of shoots, but with less growth in diameter. In fact most of the differences in total weight among the treatments can be explained by the differential growth responses of diameter and length of shoots. One exception is the significant difference in total fresh weight between treatments K_1 and NaK_1. This difference was not large, but a slightly greater reduction in growth in the NaK_1 treatment is indicated, although diameters and lengths of shoots were similar in the two treatments. There can be little doubt that the plants in each of these treatments were deficient in potassium, insofar as characteristic symptoms were indistinguishable between the two treatments and their time of incidence was the same. However, NaK_1 had a lower mortality of leaves (table III) and higher concentrations of potassium in its leaf tissues (tables V and VI). These observations indicate that NaK_1 was less deficient in potassium than K_1, despite the slightly lower yield of the former. This relatively small difference in growth perhaps does not warrant detailed consideration but it seems possible that some factor in addition to potassium deficiency was limiting growth in NaK_1. Although the sodium content of these plants was higher than that of plants from the other treatments, its concentration even in this case is not of a magnitude which would be expected to restrict growth. The effect might be ascribed to the relatively high magnesium content of tissues of this treatment. In this connection WADLEIGH and GAUCH (**48**) have shown guayule to be very sensitive to excesses of magnesium, although there are no data available which would indicate at what tissue concentration this element becomes toxic.

Within the limits of this experiment high concentrations of potassium *per se* did not result in decreased growth. At each harvest the K_3 treatment gave the highest yield, whereas NaK_3 gave much lower yield. The difference in growth between these two treatments seems related to the difference in calcium content. Further inspection of table II shows that in the interval between the two harvests, the plants of treatment K_2 made a greater increase in fresh weight than did those of K_3. From table III it may be seen that at final harvest K_3 exceeded K_2 in dry weight of mature leaves, old stems and roots, whereas K_2 excelled in weights of expanding leaves, young stems and flowers plus fruits. This evidence indicates that the higher total weight of plants in K_3 can be attributed to more rapid growth early in the experiment. However, associated with the apparent decline in rate of growth in this treatment, is the considerable decrease in calcium content of expanding leaves between the two harvests (compare tables V and VI). The calcium absorbed by these plants tended to be diluted by rapid growth. The total quantity of calcium absorbed in K_3 was comparable to quantities absorbed in NaK_1 or NaK_2 (table VII), but at final harvest the concentrations of calcium in tissues of K_3 were consid-

erably lower than were calcium concentrations in tissues of NaK_1 or NaK_2 (tables VI and VIII). Thus, in the early phases of the experiment sufficient calcium was available for maximum growth in K_3, but as the plants became larger a point was reached where the supply of calcium to the growing tissues was inadequate to maintain maximum growth rate.

Treatments K_3 and NaK_3 could not be distinguished by difference in time of incidence of the yellow-green appearance of the foliage (leaf symptoms). In this connection it is of interest that REED and HAAS (**37**) found walnut seedlings and young orange trees to be injured by high concentration of potassium only after considerable periods of growth. They reported that the foliage of the walnut seedlings eventually turned yellow, whereas the leaves of the orange trees developed characteristic yellow spots on which brown papillae appeared. WALSH and CLARKE (**50**) have reported a chlorosis of tomato associated with luxury consumption of K. Studies of lime induced chlorosis have shown in some cases a high potassium and low calcium content of chlorotic foliage (**27**).

The question of the function of sodium in plant metabolism has recently been revived and has been reviewed by RICHARDS (**39**), PIERRE and BOWER (**33**) and with particular reference to beet plants by LEHR (**26**). Beneficial results from application of sodium to plants deficient in potassium have often been reported. In this experiment little or no beneficial response to sodium was evident. As indicated above, of the two low potassium treatments, NaK_1 had a slightly lower yield of total fresh weight, although these plants had higher sodium contents than did those of other treatments. However direct comparison between the two low potassium treatments with respect to sodium response loses some force when it is considered that the calcium and magnesium contents of these plants differed.

The two series of treatments differed somewhat with respect to distribution of potassium at final harvest. Thus, the potassium concentration of leaf tissues of a given age group at a given level of potassium supply were generally higher in series 2, whereas, the concentrations of potassium in the composite samples were lower in this series (table VI). This may bear a relation to the fact that the sodium concentrations of the composite samples were higher than those of the leaf samples except in NaK_3. These observations might be taken as evidence for partial replacement of potassium by sodium in tissues other than the leaves, thereby increasing the supply of potassium to the leaves. But this is contrary to results for several crop plants where according to LEHR (**26**) it appeared that in the event of a potassium shortage most of the sodium went to the foliage, where it set free a certain quantity of potassium for use in other parts of the plant, such as the tuber of the potato, the grains of oats and barley, and the root of the beet.

Summary

1. Nursery produced guayule plants were grown in sand cultures. Two series of nutrient treatments were employed, each consisting of low, inter-

mediate and high potassium treatments. In series 1 calcium was supplied at a moderate level. Series 2 differed from series 1 in that part of the calcium was replaced by sodium in the nutrient solutions. The plants were harvested on two dates: when symptoms of potassium deficiency in the low potassium cultures were incipient, and after an additional period of growth during which effects of the treatments upon leaf symptoms and growth of the plants developed.

2. Large differences in the tissue contents of potassium were produced by variations in the potassium concentration of the nutrient solutions. Differences in potassium concentration within the plants were compensated by reciprocal differences in concentrations of other cations, thus maintaining nearly constant the total milliequivalents of cations per gram of dry matter.

3. Relatively little difference in individual or total anion contents of the plants was produced by the different treatments, although variations in the sulphate concentration of the nutrient solutions were considerable.

4. Although considerable concentrations of sodium were supplied in some of the treatments, relatively little of this element was absorbed. The concentration of sodium in the leaves was generally lower than the average concentration of this element in other parts of the plant.

5. A large proportion of the absorbed potassium, calcium, magnesium, nitrogen and sulphur was found in the leaves.

6. At given levels of potassium supply, plants from the two series of treatments contained comparable concentrations of potassium, but different concentrations of other cations. Hence a basis was provided for relating certain growth responses to concentrations of individual cations within the plants.

7. A relationship was shown between the potassium contents of the plants and growth in diameter of the shoots. Growth in length of the shoots was related to calcium contents of the plants. Rather complex effects of the treatments upon the total fresh weights of the plants could be explained largely by these two relationships.

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EFFECT OF THE ROOTSTOCK ON THE COMPOSITION OF CITRUS TREES AND FRUIT

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Introduction

In previous studies (**3**), it was shown that the amount of boron that accumulates in citrus leaves is influenced by the rootstock variety. The relative order or type of boron accumulation in the leaves of seedling trees of different varieties is the same as that impressed or imposed upon the leaves of the scion variety by these seedling varieties when used as rootstocks. This rootstock influence was found to affect many of the other inorganic constituents of Valencia orange leaves and rootlets (**4**). Recently it was pointed out (**5**) that the rootstock variety may affect the content of total phosphorus in citrus flowers.

The effect of rootstocks on fruit quality, for the most part, has dealt with the organic constituents of the fruit. As a result of a seven-year investigation on the effects of the rootstock on the composition of oranges and grapefruit (**9**), considerable data are now available regarding the total soluble solids, total and reducing sugars, pH, titratable acidity, dry matter, ash, and inorganic constituents of the peel, pulp, and juice of these fruits. Scarcely any data are available regarding the effect of the rootstock on the accumulation of inorganic constituents in the vegetative portions of lemon trees or in lemon fruit, and further data of this kind for orange and grapefruit trees and fruit are very desirable.

It is reported (**2**) that rootstocks affect the prevalence of lemon tree decline as well as the yields of fruit. In certain areas in the United States and abroad, the use of sweet orange scions on sour orange as the rootstock may be accompanied by a rapid tree decline (**10, 12**).

The present paper deals largely with differences in the inorganic composition of the peel of citrus fruits and of the leaves and rootlets of trees in which the scions (buds) of any one variety were obtained from a single parent tree and in which the seedlings used as rootstocks were carefully selected (**11**). The soil and other environmental factors were quite similar for each row of trees and frequent use was made of plot replications in order to make more accurate comparisons. The results presented in this paper add emphasis to the care required in the collection of citrus tree samples in order not to have the frequently unknown factor of rootstock variety nullify the results of careful chemical analysis. Thus in cases in which the fertilizer requirements or status of a soil are sought by means of compositional variations in various portions of citrus trees or fruit, it becomes most necessary to know that the observed chemical differences are not due instead to the nature of the rootstock varieties involved.

Methods

The chemical methods used were those previously employed (**4**). Total nitrogen was determined by the Kjeldahl method, total sulfur by weighing as barium sulfate after incineration with magnesium nitrate, whereas sugars were estimated iodometrically (**8**). In most cases only the peel of the fruit was utilized on account of the lack of sufficient drying facilities to care simultaneously for both the peel and pulp portions and the possibility of variation in the number of seeds in the pulp of some scion varieties. A sample consisting of fifteen fruits was collected from each plot tested. All of the fruit samples of a given scion variety were picked the same day. The stem and calyx (button) was discarded and each fruit was lightly scrubbed in running distilled water and wiped dry. Fresh and dry weights of whole fruit and peel were obtained. One hundred and fifty healthy mature leaves of a given scion variety were collected the same day from each plot tested. The leaves were individually washed or lightly scrubbed in running distilled water and wiped dry. Several handfuls of rootlets obtained by digging about the roots of the trees of the plots tested, were repeatedly washed by immersion in jars of tap water, followed by similar treatment with distilled water and shaking off the excess water. As any of the samples became dry in the large ventilated oven maintained at 65° C., they were finely ground in various-sized Wiley mills. Ground samples were placed in envelopes made of folded heavy brown wrapping paper and each envelope was placed in a heavy brown paper bag bearing the data descriptive of the sample. These bags were stored in an oven maintained at 50° C. and were removed temporarily during the weighing of duplicate aliquots.

In the presentation of the data the rootstock varieties are arranged in the order of their descending values. This enables one to note whether the results for the same rootstock variety plots from different locations in the orchard showed any agreement as regards their positions in the list.

Peel of navel orange fruit

The Washington navel orange fruits were collected in C.E.S. field S1 on January 29, 1946. When the calcium values were arranged (table I), the sour orange and trifoliate orange rootstock varieties were found near the top of the list, rough lemon rootstock was intermediate, whereas sweet orange and grapefruit rootstocks were lowermost. Trifoliate orange rootstock is also near the top of the list for magnesium whereas the Washington navel orange cuttings and the grapefruit rootstock are at the bottom of the list. The grapefruit (pomelo) rootstock variety being low in the list as regards both calcium and magnesium may be of interest here and in other tables because of the occurrence in Florida of magnesium deficiency especially in grapefruit trees.

When the percentages of calcium or magnesium are high, it often follows that the values for potassium may be low, as when sour orange root-

TABLE I

CALCIUM, MAGNESIUM, POTASSIUM, AND TOTAL PHOSPHORUS CONTENT OF PEEL OF WASHINGTON NAVEL ORANGE FRUITS FROM TREES ON VARIOUS ROOTSTOCKS

ROOTSTOCK VARIETY	LOCATION IN ORCHARD (C.E.S. FIELD S1)			CONTENT IN DRY MATTER
	Block	Row	Trees	
CALCIUM				
				%
Brazilian sour orange	A	43	6–10	0.864
Rubidoux sour orange	A	37	1–5	0.813
Brazilian sour orange	A	38	1–5	0.801
Trifoliate orange	B	43	11–15	0.762
Trifoliate orange	B	41	1–5	0.761
Siamese shaddock	A	40	1–5	0.734
Rubidoux sour orange	A	43	11–15	0.714
Rough lemon	A	45	11–15	0.712
Rough lemon	A	42	1–5	0.688
C.E.S. 362 sweet orange	B	38	1–5	0.685
C.E.S. 362 sweet orange	B	48	1–5	0.664
Koethen sweet orange	A	39	6–10	0.629
(None) Washington Navel orange (cuttings)	B	38	6–10	0.604
C.E.S. 343 grapefruit	A	41	1–5	0.528
MAGNESIUM				
Brazilian sour orange	A	43	6–10	0.111
C.E.S. 362 sweet orange	B	38	1–5	0.109
Trifoliate orange	B	43	11–15	0.108
Trifoliate orange	B	41	1–5	0.103
Rubidoux sour orange	A	43	11–15	0.103
Rough lemon	A	45	11–15	0.103
Koethen sweet orange	A	39	6–10	0.101
Rubidoux sour orange	A	37	1–5	0.097
Brazilian sour orange	A	38	1–5	0.097
Siamese shaddock	A	40	1–5	0.097
Rough lemon	A	42	1–5	0.094
C.E.S. 362 sweet orange	B	48	1–5	0.093
(None) Washington Navel orange (cuttings)	B	38	6–10	0.092
C.E.S. 343 grapefruit	A	41	1–5	0.076
POTASSIUM				
Trifoliate orange	B	43	11–15	0.677
C.E.S. 343 grapefruit	A	41	1–5	0.577
Trifoliate orange	B	41	1–5	0.553
Siamese shaddock	A	40	1–5	0.528
(None) Washington Navel orange (cuttings)	B	38	6–10	0.512
C.E.S. 362 sweet orange	B	48	1–5	0.511
Koethen sweet orange	A	39	6–10	0.504
Rubidoux sour orange	A	43	11–15	0.495
C.E.S. 362 sweet orange	B	38	1–5	0.404
Rubidoux sour orange	A	37	1–5	0.395
Brazilian sour orange	A	38	1–5	0.391
Brazilian sour orange	A	43	6–10	0.382
Rough lemon	A	45	11–15	0.336
Rough lemon	A	42	1–5	0.309

TABLE I (*Cont.*)

Rootstock variety	Location in orchard (C.E.S. field S1)			Content in dry matter
	Block	Row	Trees	
Total phosphorus (P)				
				p.p.m.
Trifoliate orange	B	43	11–15	770
Rubidoux sour orange	A	43	11–15	700
Trifoliate orange	B	41	1–5	690
C.E.S. 343 grapefruit	A	41	1–5	680
Siamese shaddock	A	40	1–5	630
Rubidoux sour orange	A	37	1–5	630
C.E.S. 362 sweet orange	B	48	1–5	610
Rough lemon	A	42	1–5	610
Koethen sweet orange	A	39	6–10	600
(None) Washington Navel orange (cuttings)	B	38	6–10	600
C.E.S. 362 sweet orange	B	38	1–5	590
Brazilian sour orange	A	38	1–5	580
Rough lemon	A	45	11–15	570
Brazilian sour orange	A	43	6–10	550

stocks were high in the calcium list and low in that of potassium or as when cuttings or grapefruit rootstock were low in the calcium and magnesium lists and high in that of potassium. That this reciprocal relation often does not take place is seen in table I in which trifoliate orange rootstock is near the top in each of the lists. This rootstock is reported as being a dwarfing rootstock for Marsh grapefruit (**7**) and as producing the highest soluble solids content (**6**) in citrus juice. The best quality fruit of navel orange, Valencia orange, and grapefruit was obtained (**6**) from trees on trifoliate orange rootstock although yield is a factor that cannot be overlooked. Rough lemon rootstock, which is lowest in the potassium list, is reported (**7**) as being a most vigorous rootstock and as producing the lowest soluble solids in fruit juice.

Valencia orange trees and fruit

Peel of Valencia orange fruit

The Valencia orange fruit was collected on May 24, 1945, from trees in C.E.S. field S1A. Trifoliate orange rootstock occurs near the top of the lists for calcium and magnesium values in the dry matter of the peel (table II). Duncan grapefruit rootstock was associated with the highest potassium (table III), followed by lemon shaddock, Sampson tangelo, trifoliate orange, grapefruit, and Savage citrange rootstocks. Rough lemon rootstock is low in the list and most sweet or sour orange rootstocks occur in the lower half of the list. Total phosphorus values for the peel of fruit from trees on the same rootstock in different plots lack much agreement (table IV) except in a few cases such as Duncan grapefruit and Savage

TABLE II

CALCIUM AND MAGNESIUM CONTENT OF PEEL OF VALENCIA ORANGE FRUITS FROM TREES ON VARIOUS ROOTSTOCKS

ROOTSTOCK VARIETY	LOCATION IN ORCHARD (C.E.S. FIELD S1A)		CALCIUM IN DRY MATTER	MAGNESIUM IN DRY MATTER
	Row	Trees		
			%	%
Trifoliate orange	27	11–15	0.897	0.124
Trifoliate orange	21	1–5	0.880	0.112
Standard sour orange	26	1–5	0.872	0.102
Savage citrange	23	6–10	0.864	0.109
Trifoliate orange	22	6–10	0.851	0.111
Sampson tangelo	31	6–10	0.824	0.111
African sour orange	27	1–5	0.817	0.109
Rubidoux sour orange	28	1–5	0.807	0.110
Siamese shaddock	32	1–5	0.803	0.115
Savage citrange	22	1, 2, 4, 5	0.799	0.100
Homosassa sweet orange	23	1–5	0.793	0.104
Rough lemon	30	1–5	0.792	0.115
C.E.S. 362 sweet orange	36	1–5	0.782	0.109
Rough lemon	32	6–10	0.780	0.105
Rubidoux sour orange	33	6–10	0.756	0.100
Sampson tangelo	30	6–10	0.745	0.113
C.E.S. 343 grapefruit	33	1–5	0.742	0.102
Cleopatra mandarin	31	11–15	0.740	0.108
Siamese shaddock	35	11–15	0.736	0.109
Brazilian sour orange	29	1–5	0.735	0.098
African sour orange	30	11–15	0.715	0.102
Cleopatra mandarin	32	11–15	0.713	0.111
Brazilian sour orange	29	11–15	0.708	0.099
C.E.S. 362 sweet orange	29	6–10	0.682	0.097
Koethen sweet orange	28	6–10	0.681	0.097
Standard sour orange	23	11–15	0.674	0.100
Duncan grapefruit	34	11–15	0.674	0.106
Homosassa sweet orange	27	6–10	0.672	0.098
C.E.S. 343 grapefruit	36	11–15	0.672	0.095
Duncan grapefruit	31	1–5	0.604	0.097
Koethen sweet orange	35	1–5	0.588	0.095
Lemon shaddock	33	11–13	0.539	0.116

citrange rootstocks, the latter of which headed the list. Citrange rootstocks are reported (**1**) as having improved the quality of Marsh grapefruit.

Valencia orange fruit samples also were collected on May 25, 1944, from trees in C.E.S. field S1A. The dry matter of the peel (table V) contained the highest total nitrogen content when the fruit was obtained

from trees on sour orange or rough lemon rootstocks and the lowest content when trifoliate orange was the rootstock. Total sulphur in the peel was low: trifoliate orange rootstock and Valencia orange cuttings being associated with the lowest percentages of sulfur. The reducing and total

TABLE III

POTASSIUM CONTENT OF PEEL OF VALENCIA ORANGE FRUITS FROM TREES ON VARIOUS ROOTSTOCKS

ROOTSTOCK VARIETY	LOCATION IN ORCHARD (C.E.S. FIELD S1A)		POTASSIUM IN DRY MATTER
	Row	Trees	
			%
Duncan grapefruit	31	1–5	0.826
Duncan grapefruit	34	11–15	0.754
Lemon shaddock	33	11–13	0.733
Sampson tangelo	30	6–10	0.686
Sampson tangelo	31	6–10	0.610
Trifoliate orange	21	1–5	0.598
Trifoliate orange	22	6–10	0.597
Koethen sweet orange	35	1–5	0.586
Trifoliate orange	27	11–15	0.580
C.E.S. 343 grapefruit	36	11–15	0.580
Savage citrange	23	6–10	0.553
C.E.S. 343 grapefruit	33	1–5	0.539
Siamese shaddock	35	11–15	0.531
Cleopatra mandarin	32	11–15	0.504
Siamese shaddock	32	1–5	0.493
Savage citrange	22	1,2,4,5	0.489
C.E.S. 362 sweet orange	36	1–5	0.471
Koethen sweet orange	28	6–10	0.465
Brazilian sour orange	29	11–15	0.459
Cleopatra mandarin	31	11–15	0.457
African sour orange	30	11–15	0.438
Homosassa sweet orange	27	6–10	0.437
Brazilian sour orange	29	1–5	0.435
Standard sour orange	23	11–15	0.432
C.E.S. 362 sweet orange	29	6–10	0.421
Rubidoux sour orange	28	1–5	0.397
Homosassa sweet orange	23	1–5	0.376
Standard sour orange	26	1–5	0.372
Rough lemon	32	6–10	0.363
Rubidoux sour orange	33	6–10	0.360
African sour orange	27	1–5	0.327
Rough lemon	30	1–5	0.315

sugars were highest in the case of Valencia orange cuttings and lowest when trifoliate orange was the rootstock. In the pulp the reducing sugars were highest when Siamese grapefruit and trifoliate orange were the rootstocks whereas the total sugars were highest with Valencia orange cuttings and with the Siamese grapefruit as rootstock.

ROOTLETS OF VALENCIA ORANGE TREES

The rootlets were collected on July 31, 1946 from the rootstocks of Valencia orange trees in C.E.S. field S1A. Considerable variation oc-

TABLE IV

TOTAL PHOSPHORUS CONTENT OF PEEL OF VALENCIA ORANGE FRUITS FROM TREES ON VARIOUS ROOTSTOCKS

ROOTSTOCK VARIETY	LOCATION IN ORCHARD (C.E.S. FIELD S1A)		TOTAL PHOSPHORUS (P) IN DRY MATTER
	Row	Trees	
			p.p.m.
Savage citrange	23	6–10	940
Savage citrange	22	1,2,4,5	910
Cleopatra mandarin	32	11–15	880
C.E.S. 362 sweet orange	36	1–5	860
C.E.S. 343 grapefruit	33	1–5	840
Standard sour orange	26	1–5	830
Siamese shaddock	32	1–5	830
Trifoliate orange	22	6–10	810
Standard sour orange	23	11–15	800
Rubidoux sour orange	28	1–5	800
Homosassa sweet orange	27	6–10	780
Rough lemon	30	1–5	770
African sour orange	30	11–15	760
Cleopatra mandarin	31	11–15	760
Trifoliate orange	27	11–15	760
Koethen sweet orange	35	1–5	750
Brazilian sour orange	29	1–5	740
Koethen sweet orange	28	6–10	740
Siamese shaddock	35	11–15	740
Trifoliate orange	21	1–5	740
C.E.S. 343 grapefruit	36	11–15	740
Sampson tangelo	30	6–10	740
Rubidoux sour orange	33	6–10	730
Brazilian sour orange	29	11–15	730
Duncan grapefruit	31	1–5	730
Duncan grapefruit	34	11–15	720
African sour orange	27	1–5	720
Rough lemon	32	6–10	700
C.E.S. 362 sweet orange	29	6–10	690
Sampson tangelo	31	6–10	690
Homosassa sweet orange	23	1–5	680
Lemon shaddock	33	11–13	620

curred in the thickness of the rootlets and only the young and small-sized rootlets were taken. Trifoliate orange, Savage citrange, and Brazilian sour orange rootstocks occur in the upper portion of the list of calcium values (table VI) whereas Sampson tangelo and Koethen sweet orange rootstocks occur in the lower portion, the two portions of the list being markedly different in calcium values. In table VI it is seen that the Sampson tangelo rootstock is relatively low and the trifoliate orange rootstock relatively high in the list of values for the various constituents.

Marsh grapefruit trees and fruit

PEEL OF MARSH GRAPEFRUIT

The fruit samples were collected on May 26, 1945, from trees in C.E.S. field S1. In the list of values (table VII) for calcium the sour orange root-

stocks rank fairly high whereas rough lemon and lemon shaddock rootstocks rank lower. All of the grapefruit rootstocks occur in the upper half of the list of the values for magnesium. Grapefruit and lemon shaddock rootstocks rank high in the list of potassium values whereas sour orange

TABLE V

TOTAL NITROGEN, TOTAL SULPHUR, REDUCING AND TOTAL (AS REDUCING) SUGARS IN VALENCIA ORANGE FRUIT FROM TREES ON VARIOUS ROOTSTOCKS

ROOTSTOCK VARIETY	LOCATION IN ORCHARD (C.E.S. FIELD S1A)		CONTENT IN DRY MATTER
	Row	Trees	
TOTAL NITROGEN IN PEEL			
			%
African sour orange	27	1–5	1.204
Brazilian sour orange	29	1–5	1.126
Rough lemon	30	1–5	1.042
C.E.S. 362 sweet orange	36	1–5	0.987
Siamese grapefruit	32	1–5	0.948
(None) Valencia orange (cuttings)	18	6–10	0.930
Koethen sweet orange	35	1–5	0.840
Duncan grapefruit	34	11–15	0.825
Trifoliate orange	21	1–5	0.803
REDUCING SUGARS IN PEEL			
(None) Valencia orange (cuttings)	18	6–10	16.08
Rough lemon	30	1–5	14.00
Koethen sweet orange	35	1–5	13.96
Siamese grapefruit	32	1–5	13.58
C.E.S. 362 sweet orange	36	1–5	13.38
Brazilian sour orange	29	1–5	13.30
African sour orange	27	1–5	13.30
Duncan grapefruit	34	11–15	11.28
Trifoliate orange	21	1–5	8.78
TOTAL (AS REDUCING) SUGARS IN PEEL			
(None) Valencia orange (cuttings)	18	6–10	27.52
Siamese grapefruit	32	1–5	24.86
C.E.S. 362 sweet orange	36	1–5	24.72
Rough lemon	30	1–5	24.40
Koethen sweet orange	35	1–5	24.16
Duncan grapefruit	34	11–15	23.39
Brazilian sour orange	29	1–5	23.17
African sour orange	27	1–5	22.84
Trifoliate orange	21	1–5	17.25

ROOTSTOCK VARIETY	CONTENT IN DRY MATTER
TOTAL SULPHUR IN PEEL	
	%
Rough lemon	0.082
Koethen sweet orange	0.078
Duncan grapefruit	0.069
Brazilian sour orange	0.065
African sour orange	0.062
Siamese grapefruit	0.055
C.E.S. 362 sweet orange	0.051
Trifoliate orange	0.038
(None) Valencia orange (cuttings)	0.027
REDUCING SUGARS IN PULP	
Siamese grapefruit	32.00
Trifoliate orange	29.35
C.E.S. 362 sweet orange	28.75
(None) Valencia orange (cuttings)	28.55
Rough lemon	28.40
Brazilian sour orange	28.40
Duncan grapefruit	26.95
African sour orange	26.85
Koethen sweet orange	25.80
TOTAL (AS REDUCING) SUGARS IN PULP	
(None) Valencia orange (cuttings)	43.40
Siamese grapefruit	42.90
Koethen sweet orange	42.80
Trifoliate orange	41.50
Brazilian sour orange	41.20
C.E.S. 362 sweet orange	40.60
Rough lemon	39.10
African sour orange	37.00
Duncan grapefruit	36.00

and sweet orange rootstocks occur in the lower half of the list. The total phosphorus values are low in the cases of rough lemon and lemon shaddock rootstocks. Trifoliate orange rootstocks were conspicuous for their lack of agreement in table VII although fair agreement of values were obtained for rootstocks of a given variety even though occurring in widely scattered rows or plots.

TABLE VI

CALCIUM, MAGNESIUM, POTASSIUM, AND TOTAL PHOSPHORUS CONTENT OF ROOTLETS FROM VALENCIA ORANGE TREES ON VARIOUS ROOTSTOCKS

ROOTSTOCK VARIETY	LOCATION IN ORCHARD (C.E.S. FIELD S1A)		CONTENT IN DRY MATTER
	Row	Trees	
CALCIUM			
			%
Trifoliate orange	27	11–15	2.723
Savage citrange	23	6–10	2.594
Trifoliate orange	22	6–10	2.544
Brazilian sour orange	29	11–15	2.281
Savage citrange	22	4–5	2.243
Brazilian sour orange	29	1–5	2.200
Sampson tangelo	30	6–10	1.363
Sampson tangelo	31	6–10	1.222
Koethen sweet orange	28	6–10	1.064
Koethen sweet orange	35	1–5	0.886
MAGNESIUM			
Koethen sweet orange	28	6–10	0.343
Trifoliate orange	27	11–15	0.317
Brazilian sour orange	29	11–15	0.298
Savage citrange	23	6–10	0.265
Trifoliate orange	22	6–10	0.249
Koethen sweet orange	35	1–5	0.228
Sampson tangelo	31	6–10	0.175
Sampson tangelo	30	6–10	0.168
Savage citrange	22	4–5	0.166
POTASSIUM			
Koethen sweet orange	28	6–10	1.661
Savage citrange	23	6–10	1.550
Trifoliate orange	22	6–10	1.425
Trifoliate orange	27	11–15	1.416
Brazilian sour orange	29	1–5	1.364
Koethen sweet orange	35	1–5	1.218
Savage citrange	22	4–5	1.098
Sampson tangelo	30	6–10	1.038
Sampson tangelo	31	6–10	0.832
Brazilian sour orange	29	11–15	0.801
TOTAL PHOSPHORUS (P)			
			p.p.m.
Trifoliate orange	27	11–15	1360
Trifoliate orange	22	6–10	1270
Savage citrange	23	6–10	1230
Koethen sweet orange	28	6–10	1180
Cleopatra mandarin	32	11–15	1090
Rough lemon	30	1–5	1070
Duncan grapefruit	31	1–5	1010
Brazilian sour orange	29	1–5	970
Duncan grapefruit	34	11–15	900
Rough lemon	32	6–10	890
Savage citrange	22	4–5	820
Koethen sweet orange	35	1–5	810
Cleopatra mandarin	31	11–15	810
Brazilian sour orange	29	11–15	770
Sampson tangelo	30	6–10	770
Sampson tangelo	31	6–10	720

TABLE VII

CALCIUM, MAGNESIUM, POTASSIUM, AND TOTAL PHOSPHORUS CONTENT OF PEEL OF MARSH GRAPEFRUIT FROM TREES ON VARIOUS ROOTSTOCKS

ROOTSTOCK VARIETY	LOCATION IN ORCHARD (C.E.S. FIELD S1)		CONTENT IN DRY MATTER
	Row	Trees	
CALCIUM			
			%
Trifoliate orange	33	6–10	0.637
Homosassa sweet orange	29	6–10	0.587
Duncan grapefruit	20	1–5	0.582
Brazilian sour orange	28	6–10	0.573
African sour orange	27	6–10	0.565
African sour orange	34	1–5	0.565
Brazilian sour orange	35	1–5	0.557
C.E.S. 362 sweet orange	20	6–10	0.541
Camulos grapefruit	17	1–5	0.541
C.E.S. 362 sweet orange	37	1–5	0.537
Lemon shaddock	32	6–10	0.536
Rough lemon	22	1–5	0.535
Camulos grapefruit	16	1–5	0.533
Homosassa sweet orange	36	1–5	0.490
Rough lemon	37	6–10	0.478
Trifoliate orange	23	1–5	0.472
Lemon shaddock	21	1–5	0.430
MAGNESIUM			
Homosassa sweet orange	29	6–10	0.099
Trifoliate orange	33	6–10	0.098
Lemon shaddock	21	1–5	0.089
C.E.S. 362 sweet orange	37	1–5	0.087
Camulos grapefruit	16	1–5	0.086
Brazilian sour orange	35	1–5	0.083
Duncan grapefruit	20	1–5	0.082
Camulos grapefruit	17	1–5	0.081
Brazilian sour orange	28	6–10	0.081
African sour orange	27	6–10	0.081
Rough lemon	37	6–10	0.078
C.E.S. 362 sweet orange	20	6–10	0.077
Homosassa sweet orange	36	1–5	0.077
African sour orange	34	1–5	0.075
Lemon shaddock	32	6–10	0.075
Trifoliate orange	23	1–5	0.072
Rough lemon	22	1–5	0.069
POTASSIUM			
Camulos grapefruit	16	1–5	0.861
Lemon shaddock	21	1–5	0.833
Lemon shaddock	32	6–10	0.833
Duncan grapefruit	20	1–5	0.786
Camulos grapefruit	17	1–5	0.768
Trifoliate orange	23	1–5	0.746
C.E.S. 362 sweet orange	37	1–5	0.700
Rough lemon	37	6–10	0.666
Brazilian sour orange	35	1–5	0.665
Homosassa sweet orange	36	1–5	0.658
C.E.S. 362 sweet orange	20	6–10	0.625
Homosassa sweet orange	29	6–10	0.615
Brazilian sour orange	28	6–10	0.574
African sour orange	34	1–5	0.564
African sour orange	27	6–10	0.536
Trifoliate orange	33	6–10	0.497
Rough lemon	22	1–5	0.444

TABLE VII (*Cont.*)

Rootstock variety	Location in orchard (C.E.S. field S1)		Content in dry matter
	Row	Trees	
Total phosphorus (P)			
			p.p.m.
Homosassa sweet orange	36	1–5	850
Camulos grapefruit	16	1–5	840
Camulos grapefruit	17	1–5	830
C.E.S. 362 sweet orange	20	6–10	830
C.E.S. 362 sweet orange	37	1–5	820
Duncan grapefruit	20	1–5	810
Brazilian sour orange	28	6–10	800
Brazilian sour orange	35	1–5	770
African sour orange	27	6–10	760
Trifoliate orange	23	1–5	750
Marsh grapefruit	29	6–10	740
Lemon shaddock	21	1–5	740
Rough lemon	37	6–10	730
African sour orange	34	1–5	710
Rough lemon	22	1–5	680
Lemon shaddock	32	6–10	650
Trifoliate orange	33	6–10	620

Leaves of Marsh grapefruit trees on various rootstocks

The leaf samples were collected on August 7, 1945, from trees in block B (with the exception of row 35 in block C) in C.E.S. field S1. Table VIII clearly shows that the dry matter of the leaves of trees on trifoliate orange or sour orange rootstocks contained more calcium than that of leaves of trees on sweet orange, grapefruit, rough lemon, or lemon shaddock rootstocks. High magnesium values in Marsh grapefruit leaves was associated with the rough lemon rootstock whereas low values were associated with the grapefruit rootstock. This may be of interest in situations in Florida where magnesium deficiency is prevalent over considerable areas. The grapefruit rootstock was associated with high potassium values. Lemon shaddock, trifoliate orange and Brazilian sour orange rootstocks were associated with low percentages of total phosphorus.

Leaves of Marsh grapefruit trees on various grapefruit rootstocks

All of these leaf samples were collected on February 26, 1947, from trees on grapefruit rootstocks in C.E.S. field S1. From the data (table IX) it appears that the percentages of calcium, magnesium, and potassium in the leaves of Marsh grapefruit trees vary according to the grapefruit rootstock variety. These results may be of considerable importance for trees on Florida soils.

Rootlets of Marsh grapefruit trees

The rootlets were collected on March 5, 1947, from trees in block B (with a few samples in block C) in C.E.S. field S1. The calcium percen-

TABLE VIII

CALCIUM, MAGNESIUM, POTASSIUM, AND TOTAL PHOSPHORUS CONTENT OF MATURE MARSH GRAPEFRUIT LEAVES FROM TREES ON VARIOUS ROOTSTOCKS

ROOTSTOCK VARIETY	LOCATION IN ORCHARD (C.E.S. FIELD S1, B AND C*)		CONTENT IN DRY MATTER
	ROW	TREES	
CALCIUM			
			%
Brazilian sour orange	35*	1–5	6.748
African sour orange	34	1–5	6.513
Trifoliate orange	23	1–5	6.442
Brazilian sour orange	28	6–10	6.199
Trifoliate orange	33	6–10	6.181
African sour orange	27	6–10	5.916
C.E.S. 362 sweet orange	37	1–5	5.866
Homosassa sweet orange	36	1–5	5.571
Duncan grapefruit	20	1–5	5.557
Homosassa sweet orange	29	6–10	5.457
Camulos grapefruit	16	1–5	5.393
Camulos grapefruit	17	1–5	5.383
Rough lemon	37	6–10	5.319
C.E.S. 362 sweet orange	20	6–10	5.270
Lemon shaddock	21	1–5	4.927
Rough lemon	22	1–5	4.795
Lemon shaddock	32	6–10	4.792
MAGNESIUM			
Rough lemon	37	6–10	0.555
Trifoliate orange	33	6–10	0.520
Lemon shaddock	32	6–10	0.495
Homosassa sweet orange	29	6–10	0.468
Rough lemon	22	1–5	0.452
Homosassa sweet orange	36	1–5	0.451
African sour orange	34	1–5	0.440
African sour orange	27	6–10	0.439
Brazilian sour orange	35*	1–5	0.430
C.E.S. 362 sweet orange	37	1–5	0.427
C.E.S. 362 sweet orange	20	6–10	0.426
Trifoliate orange	23	1–5	0.426
Lemon shaddock	21	1–5	0.425
Brazilian sour orange	28	6–10	0.400
Camulos grapefruit	17	1–5	0.380
Duncan grapefruit	20	1–5	0.350
Camulos grapefruit	16	1–5	0.326
POTASSIUM			
Camulos grapefruit	16	1–5	1.024
Duncan grapefruit	20	1–5	0.910
Camulos grapefruit	17	1–5	0.711
Lemon shaddock	21	1–5	0.680
Rough lemon	22	1–5	0.610
Homosassa sweet orange	36	1–5	0.590
African sour orange	27	6–10	0.578
C.E.S. 362 sweet orange	37	1–5	0.562
Lemon shaddock	32	6–10	0.560
C.E.S. 362 sweet orange	20	6–10	0.544
Rough lemon	37	6–10	0.512
Brazilian sour orange	28	6–10	0.506
Trifoliate orange	23	1–5	0.503
Homosassa sweet orange	29	6–10	0.496
Brazilian sour orange	35*	1–5	0.446
Trifoliate orange	33	6–10	0.439
African sour orange	34	1–5	0.410

TABLE VIII (*Cont.*)

Rootstock variety	Location in orchard (C.E.S. field S1, B and C*)		Content in dry matter
	Row	Trees	
Total phosphorus (P)			
			p.p.m.
Rough lemon	22	1–5	1400
Duncan grapefruit	20	1–5	1290
African sour orange	27	6–10	1200
C.E.S. 362 sweet orange	37	1–5	1190
C.E.S. 362 sweet orange	20	6–10	1170
Rough lemon	37	6–10	1160
Camulos grapefruit	17	1–5	1160
African sour orange	34	1–5	1150
Homosassa sweet orange	36	1–5	1140
Camulos grapefruit	16	1–5	1060
Lemon shaddock	21	1–5	1050
Homosassa sweet orange	29	6–10	1020
Trifoliate orange	33	6–10	1010
Trifoliate orange	23	1–5	960
Brazilian sour orange	35*	1–5	960
Lemon shaddock	32	6–10	910
Brazilian sour orange	28	6–10	890

tages in the rootlets of grapefruit trees on various rootstocks (table X) were high when trifoliate orange and sour orange rootstocks were used, which is in agreement with the results obtained with Valencia orange on these rootstocks (table VI). Considerable variation occurs in the rootlets of other rootstock varieties such as grapefruit. A comparison of the relative order of the grapefruit rootstock varieties in the list of percentages for magnesium (tables IX and X) suggests that the order is approximately the same in both the rootlets and Marsh grapefruit leaves. Table X indicates the wide variation that occurs in the potassium content of the rootlets of Marsh grapefruit trees on various grapefruit rootstock varieties. The rootlets of Marsh grapefruit trees are high in calcium and low in potassium when the rootstock is sour orange and are high in both calcium and potassium when the rootstock is trifoliate orange.

Eureka lemon trees and fruit

Peel of Eureka lemon fruit

The lemon fruit in the silver stage of maturity were collected on November 28, 1945, from trees in C.E.S. field 3. Data for the effect of rootstocks on the inorganic content of lemon peel is shown in table XI. Sour orange and rough lemon rootstocks were associated with high calcium percentages in the dry matter of the peel and sweet lemon rootstock with low percentages. The peel of fruits of trees on sour orange, sweet orange, or pomelo rootstocks contained low percentages of magnesium. The range of the potassium content of lemon peel extended from 0.475 to 1.136 per cent accord-

ing to the rootstock variety. Pomelo and tangelo rootstocks were highest in the list of potassium values whereas sweet lemon, rough lemon, and sour orange rootstocks were lowest. The pomelo rootstocks also were high in the list of values for total phosphorus.

TABLE IX

CALCIUM, MAGNESIUM, AND POTASSIUM CONTENT OF MATURE MARSH GRAPEFRUIT LEAVES FROM TREES ON VARIOUS GRAPEFRUIT ROOTSTOCKS

GRAPEFRUIT ROOTSTOCK VARIETY	LOCATION IN ORCHARD (C.E.S. FIELD S1, B AND C)			CONTENT IN DRY MATTER
	Block	Row	Trees	
CALCIUM				
				%
Camulos	B	16	1–5	4.879
Camulos	B	17	1–5	4.741
Pernambuco	C	28	6–10	4.721
Camulos	B	13	1–5	4.707
Pernambuco	C	27	6–10	4.643
Duncan	B	20	1–5	4.614
McCarty	C	24	6–10*	4.577
(None) Marsh (cuttings)	B	18	1–10	4.564
McCarty	C	23	6–10	4.410
Tresca	C	25	6–10	4.366
Tresca	C	26	6–10	4.346
(None) Marsh (cuttings)	B	19	6–10	4.341
MAGNESIUM				
Tresca	C	25	6–10	0.365
Tresca	C	26	6–10	0.362
Camulos	B	17	1–5	0.297
Pernambuco	C	27	6–10	0.287
Pernambuco	C	28	6–10	0.281
Camulos	B	16	1–5	0.272
Camulos	B	13	1–5	0.264
McCarty	C	24	6–10	0.264
(None) Marsh (cuttings)	B	19	6–10	0.262
(None) Marsh (cuttings)	B	18	1–10	0.260
Duncan	B	20	1–5	0.253
McCarty	C	23	6–10	0.252
POTASSIUM				
(None) Marsh (cuttings)	B	19	6–10	0.760
Duncan	B	20	1–5	0.704
Pernambuco	C	27	6–10	0.690
(None) Marsh (cuttings)	B	18	1–10	0.689
McCarty	C	23	6–10	0.680
Pernambuco	C	28	6–10	0.665
Camulos	B	13	1–5	0.651
McCarty	C	24	6–10	0.626
Camulos	B	16	1–5	0.622
Camulos	B	17	1–5	0.547
Tresca	C	25	6–10	0.521
Tresca	C	26	6–10	0.443

LEAVES OF EUREKA LEMON TREES

Healthy mature Eureka lemon leaves were collected on July 18, 1945, from trees in C.E.S. field 3. The highest calcium content in the dry matter

of lemon leaves (table XII) was in those from trees on Cleopatra mandarin rootstock whereas the lowest was in leaves from trees on sweet lemon rootstock. The first ten rootstocks in the list for magnesium included: rough

TABLE X

CALCIUM, MAGNESIUM, AND POTASSIUM CONTENT OF ROOTLETS FROM MARSH GRAPEFRUIT TREES ON VARIOUS ROOTSTOCKS

ROOTSTOCK VARIETY	LOCATION IN ORCHARD (C.E.S. FIELD S1, B AND C*)		CONTENT IN DRY MATTER
	Row	Trees	
CALCIUM			
			%
Trifoliate orange	23	1–5	2.517
African sour orange	34	1–5	2.317
Brazilian sour orange	35	1–5	2.211
Tresca grapefruit	25*	6–10	2.009
Pernambuco grapefruit	27*	6–10	1.877
Rough lemon	22	1–5	1.658
Lemon shaddock	21	1–5	1.609
Camulos grapefruit	16	1–5	1.568
(None) Marsh grapefruit (cuttings)	18	1–5	1.411
Homosassa sweet orange	36	1–5	1.410
McCarty grapefruit	23*	6–10	1.348
C.E.S. 362 sweet orange	37	1–5	1.226
Duncan grapefruit	20	1–5	1.194
MAGNESIUM			
Homosassa sweet orange	36	1–5	0.382
Lemon shaddock	21	1–5	0.326
Camulos grapefruit	16	1–5	0.306
C.E.S. 362 sweet orange	37	1–5	0.284
Tresca grapefruit	25*	6–10	0.279
Pernambuco grapefruit	27*	6–10	0.276
Brazilian sour orange	35	1–5	0.271
(None) Marsh grapefruit (cuttings)	18	1–5	0.264
African sour orange	34	1–5	0.247
Trifoliate orange	23	1–5	0.226
Rough lemon	22	1–5	0.213
McCarty grapefruit	23*	6–10	0.196
Duncan grapefruit	20	1–5	0.181
POTASSIUM			
Trifoliate orange	23	1–5	1.815
Pernambuco grapefruit	27*	6–10	1.423
Tresca grapefruit	25*	6–10	1.412
(None) Marsh grapefruit (cuttings)	18	1–5	1.161
Camulos grapefruit	16	1–5	1.006
Rough lemon	22	1–5	0.967
Lemon shaddock	21	1–5	0.836
McCarty grapefruit	23*	6–10	0.724
Homosassa sweet orange	36	1–5	0.688
Duncan grapefruit	20	1–5	0.686
C.E.S. 362 sweet orange	37	1–5	0.602
Brazilian sour orange	35	1–5	0.537
African sour orange	34	1–5	0.499

lemon, Cleopatra mandarin, Sampson tangelo, sweet lemon, and Madame Vinus rootstock, whereas the lowest eleven rootstocks included: various sour orange, various sweet orange, and pomelo rootstocks. Pomelo and

Sampson tangelo rootstocks are associated with the highest potassium values in lemon leaves whereas rough lemon, sweet lemon, sour orange, and Cleopatra mandarin rootstocks are associated with relatively low potassium values. Pomelo, tangelo, and sweet orange rootstocks are high in the list of total phosphorus values whereas rough lemon and sour orange rootstocks are low.

TABLE XI

CALCIUM, MAGNESIUM, POTASSIUM, AND TOTAL PHOSPHORUS CONTENT OF PEEL OF EUREKA LEMON FRUITS FROM TREES ON VARIOUS ROOTSTOCKS

ROOTSTOCK VARIETY	LOCATION IN ORCHARD (C.E.S. FIELD 3)			CONTENT IN DRY MATTER
	Block	Row	Trees	
CALCIUM				
				%
Brazilian sour orange	C	15	1–5	1.545
Rubidoux sour orange	C	11	1–5	1.481
Brazilian sour orange	B	28	1–5	1.353
Rubidoux sour orange	B	29	1–5	1.286
Rough lemon	C	14	1–5	1.249
Rough lemon	B	26	1–5	1.224
Cleopatra mandarin	B	35	6–10	1.114
C.E.S. 343 pomelo	B	36	6–10	1.093
Bessie sweet orange	B	10	1–5	1.087
Koethen sweet orange	B	16	1–5	1.071
Madame Vinus	B	28	6–10	1.040
Madame Vinus	B	13	1–5	1.040
Sampson tangelo	C	23	11–15	1.039
Duncan pomelo	B	23	1–5	1.032
Cleopatra mandarin	C	13	6–10	1.024
C.E.S. 343 pomelo	B	21	1–5	1.021
Bessie sweet orange	C	12	1–5	0.985
Sweet lemon	C	17	6–10	0.977
Sampson tangelo	B	38	6–10	0.955
Sweet lemon	C	34	11–15	0.944
MAGNESIUM				
Rough lemon	C	14	1–5	0.156
Sampson tangelo	C	23	11–15	0.143
Sweet lemon	C	34	11–15	0.135
Rough lemon	B	26	1–5	0.133
Sampson tangelo	B	38	6–10	0.131
Sweet lemon	C	17	6–10	0.127
Cleopatra mandarin	B	35	6–10	0.124
Bessie sweet orange	B	10	1–5	0.122
Cleopatra mandarin	C	13	6–10	0.121
Madame Vinus	B	28	6–10	0.121
Brazilian sour orange	C	15	1–5	0.116
Rubidoux sour orange	C	11	1–5	0.115
Madame Vinus	B	13	1–5	0.114
Rubidoux sour orange	B	29	1–5	0.109
Koethen sweet orange	B	16	1–5	0.105
C.E.S. 343 pomelo	B	36	6–10	0.105
Bessie sweet orange	C	12	1–5	0.104
Brazilian sour orange	B	28	1–5	0.104
Duncan pomelo	B	23	1–5	0.099
C.E.S 343 pomelo	B	21	1–5	0.093

TABLE XI (*Cont.*)

Rootstock variety	Location in orchard (C.E.S. field 3)			Content in dry matter
	Block	Row	Trees	
Potassium				
Duncan pomelo	B	23	1–5	1.136
C.E.S 343 pomelo	B	21	1–5	0.965
Sampson tangelo	C	23	11–15	0.911
Sampson tangelo	B	38	6–10	0.903
Madame Vinus	B	28	6–10	0.885
C.E.S 343 pomelo	B	36	6–10	0.865
Cleopatra mandarin	B	35	6–10	0.787
Koethen sweet orange	B	16	1–5	0.777
Madame Vinus	B	13	1–5	0.761
Rubidoux sour orange	B	29	1–5	0.742
Bessie sweet orange	B	10	1–5	0.726
Cleopatra mandarin	C	13	6–10	0.721
Bessie sweet orange	C	12	1–5	0.718
Brazilian sour orange	B	28	1–5	0.696
Rubidoux sour orange	C	11	1–5	0.628
Brazilian sour orange	C	15	1–5	0.568
Rough lemon	B	26	1–5	0.517
Sweet lemon	C	17	6–10	0.512
Rough lemon	C	14	1–5	0.485
Sweet lemon	C	34	11–15	0.475
Total phosphorus (P)				
				p.p.m.
Sampson tangelo	C	23	11–15	840
Duncan pomelo	B	23	1–5	830
Cleopatra mandarin	B	35	6–10	720
C.E.S 343 pomelo	B	21	1–5	710
C.E.S. 343 pomelo	B	36	6–10	670
Madame Vinus	B	13	1–5	670
Brazilian sour orange	B	28	1–5	660
Rubidoux sour orange	B	29	1–5	640
Sweet lemon	C	17	6–10	640
Sampson tangelo	B	38	6–10	640
Cleopatra mandarin	C	13	6–10	630
Madame Vinus	B	28	6–10	620
Bessie sweet orange	B	10	1–5	620
Bessie sweet orange	C	12	1–5	620
Rough lemon	B	26	1–5	610
Rough lemon	C	14	1–5	590
Brazilian sour orange	C	15	1–5	590
Bessie sweet orange	B	16	1–5	580
Sweet lemon	C	34	11–15	580
Rubidoux sour orange	C	11	1–5	580

Rootlets of Eureka lemon trees

Rootlets were dug on August 29, 1946, from the rootstocks of lemon trees in C.E.S. field 3. The number of samples is limited and there are no additional data available. The rootlets of Siamese grapefruit and African sour orange rootstocks (table XIII) contained the most calcium whereas those of rough lemon and Sampson tangelo contained the least. The rootlets of Koethen sweet orange, Siamese grapefruit and rough lemon root-

stocks contained the most magnesium, whereas those of African sour orange and Sampson tangelo rootstocks contained the least. Rootlets of these latter rootstocks also contained the least potassium, whereas those of Cunningham citrange and rough lemon contained the most potassium and most total phosphorus.

TABLE XII

CALCIUM, MAGNESIUM, POTASSIUM, AND TOTAL PHOSPHORUS CONTENT OF MATURE EUREKA LEMON LEAVES FROM TREES ON VARIOUS ROOTSTOCKS

ROOTSTOCK VARIETY	LOCATION IN ORCHARD (C.E.S. FIELD 3)			CONTENT IN DRY MATTER
	Block	Row	Trees	
CALCIUM				
				%
Cleopatra mandarin	C	13	6–10	5.875
Cleopatra mandarin	B	35	6–10	5.661
Bessie sweet orange	B	10	1–5	5.410
Sampson tangelo	C	23	11–15	5.272
Bessie sweet orange	C	12	1–5	5.210
Madame Vinus	C	13	1–5	5.126
Rubidoux sour orange	C	11	1–5	5.102
Bessie sweet orange	C	12	1–5	5.076
Sampson tangelo	B	38	6–10	5.071
Duncan pomelo	B	23	1–5	5.023
Brazilian sour orange	C	15	1–5	4.986
Madame Vinus	B	28	6–10	4.941
C.E.S. 343 Pomelo	B	21	1–5	4.813
Brazilian sour orange	B	28	1–5	4.708
Rough lemon	C	14	1–5	4.704
Koethen sweet orange	B	16	1–5	4.668
C.E.S. 343 pomelo	B	36	6–10	4.526
Rough lemon	B	26	1–5	4.436
Rubidoux sour orange	B	29	1–5	4.269
Sweet lemon	C	17	6–10	4.182
Sweet lemon	C	34	11–15	4.182
MAGNESIUM				
Rough lemon	C	14	1–5	0.512
Cleopatra mandarin	C	13	6–10	0.443
Sampson tangelo	C	23	11–15	0.436
Sweet lemon	C	17	6–10	0.430
Sampson tangelo	B	38	6–10	0.424
Rough lemon	B	26	1–5	0.405
Cleopatra mandarin	B	35	6–10	0.382
Madame Vinus	B	28	6–10	0.377
Sweet lemon	C	34	11–15	0.366
Madame Vinus	C	13	1–5	0.364
Brazilian sour orange	C	15	1–5	0.350
Bessie sweet orange	B	10	1–5	0.339
Bessie sweet orange	C	12	1–5	0.329
Bessie sweet orange	C	12	1–5	0.322
Koethen sweet orange	B	16	1–5	0.304
Rubidoux sour orange	C	11	1–5	0.297
C.E.S. 343 pomelo	B	36	6–10	0.285
Brazilian sour orange	B	28	1–5	0.283
C.E.S. 343 pomelo	B	21	1–5	0.280
Rubidoux sour orange	B	29	1–5	0.256
Duncan pomelo	B	23	1–5	0.220

TABLE XII (*Cont.*)

Rootstock variety	Location in orchard (C.E.S. field 3)			Content in dry matter
	Block	Row	Trees	
Potassium				
Duncan pomelo	B	23	1–5	1.073
C.E.S. 343 pomelo	B	21	1–5	1.036
C.E.S. 343 pomelo	B	36	6–10	0.947
Sampson tangelo	B	38	6–10	0.831
Sampson tangelo	C	23	11–15	0.732
Koethen sweet orange	B	16	1–5	0.685
Madame Vinus	C	13	1–5	0.645
Bessie sweet orange	C	12	1–5	0.644
Bessie sweet orange	C	12	1–5	0.603
Rubidoux sour orange	B	29	1–5	0.602
Bessie sweet orange	B	10	1–5	0.564
Madame Vinus	B	28	6–10	0.479
Cleopatra mandarin	C	13	6–10	0.476
Cleopatra mandarin	B	35	6–10	0.463
Sweet lemon	C	17	6–10	0.462
Brazilian sour orange	B	28	1–5	0.454
Brazilian sour orange	C	15	1–5	0.448
Rubidoux sour orange	C	11	1–5	0.447
Sweet lemon	C	34	11–15	0.428
Rough lemon	B	26	1–5	0.407
Rough lemon	C	14	1–5	0.306
Total phosphorus (P)				
				p.p.m.
C.E.S. 343 pomelo	B	21	1–5	1300
Sampson tangelo	C	23	11–15	1280
Koethen sweet orange	B	16	1–5	1120
Duncan pomelo	B	23	1–5	1090
Bessie sweet orange	B	10	1–5	1090
C.E.S. 343 pomelo	B	36	6–10	1080
Sweet lemon	C	17	6–10	1080
Bessie sweet orange	C	12	1–5	1070
Sampson tangelo	B	38	6–10	1050
Madame Vinus	C	13	1–5	1050
Rubidoux sour orange	B	29	1–5	990
Cleopatra mandarin	C	35	6–10	970
Sweet lemon	C	34	11–15	960
Madame Vinus	B	28	6–10	950
Cleopatra mandarin	C	13	6–10	950
Rough lemon	B	26	1–5	890
Rubidoux sour orange	C	11	1–5	800
Rough lemon	C	14	1–5	760
Brazilian sour orange	B	28	1–5	760
Brazilian sour orange	C	15	1–5	700

Summary and conclusions

The peel of Washington Navel orange fruit contained high percentages of calcium when the trees were on sour orange or on trifoliate orange rootstocks and low calcium values when on sweet orange or grapefruit rootstocks. Magnesium values were lowest when grapefruit rootstock was used. The potassium percentages in navel orange peel were highest for trifoliate orange and grapefruit rootstocks, followed in descending order

by sweet orange, sour orange, and rough lemon rootstocks. Trifoliate orange rootstock ranked high as regards the calcium, magnesium, potassium, and total phosphorus values in the peel of navel oranges.

In Valencia orange peel the calcium content was high when the rootstocks were Duncan grapefruit, lemon shaddock, Sampson tangelo and trifoliate orange and low when the rootstocks were sour orange or rough

TABLE XIII

CALCIUM, MAGNESIUM, POTASSIUM, AND TOTAL PHOSPHORUS CONTENT OF ROOTLETS FROM EUREKA LEMON TREES ON VARIOUS ROOTSTOCKS

ROOTSTOCK VARIETY	CONTENT IN DRY MATTER
CALCIUM	
	%
Siamese grapefruit	2.458
African sour orange	2.352
Cunningham citrange	2.157
Koethen sweet orange	1.966
Rough lemon	1.742
Sampson tangelo	1.430
MAGNESIUM	
Koethen sweet orange	0.392
Siamese grapefruit	0.375
Rough lemon	0.355
Cunningham citrange	0.291
African sour orange	0.261
Sampson tangelo	0.220
POTASSIUM	
Cunningham citrange	1.990
Rough lemon	1.598
Koethen sweet orange	1.118
African sour orange	0.859
Sampson tangelo	0.842
TOTAL PHOSPHORUS (P)	
	p.p.m.
Rough lemon	1270
Cunningham citrange	1100
Sampson tangelo	890
Koethen sweet orange	880
African sour orange	720
Siamese grapefruit	690

lemon. The potassium percentages when the trees were on Duncan grapefruit rootstock were double those found when rough lemon rootstock was used. Duncan grapefruit, lemon shaddock, Sampson tangelo, trifoliate orange, grapefruit, and Savage citrange rootstocks were associated with high potassium values and rough lemon rootstock with low values. Most sweet orange and sour orange rootstocks occurred in the lower portion of the list of potassium values. The total phosphorus content was highest

when the rootstock was Savage citrange. The peel of Valencia orange contained high total nitrogen with sour orange and low total nitrogen with trifoliate orange rootstocks. Sugars were usually high in peel and pulp when Valencia orange cuttings or Siamese grapefruit rootstock was used. Sugars were very low in the peel and high in the pulp when the rootstock variety was trifoliate orange. The trifoliate orange rootstock was associated with high values for calcium, magnesium, potassium, and total phosphorus in the rootlets of Valencia orange trees, whereas Sampson tangelo rootstock was associated with low values.

The calcium percentages were high in the peel of Marsh grapefruit when sour orange rootstock was used and low when rough lemon and lemon shaddock rootstocks were used. The magnesium values were rather high when grapefruit rootstocks were used. The peel contained high potassium values in fruits of trees on grapefruit and lemon shaddock rootstocks and lower values for trees on sour orange and sweet orange rootstocks.

The leaves of Marsh grapefruit trees contained higher percentages of calcium when the rootstocks were trifoliate orange or sour orange and lower values when the rootstocks were sweet orange, grapefruit, rough lemon, or lemon shaddock. In contrast to the magnesium content in the peel of Marsh grapefruit, the leaves of grapefruit trees on grapefruit rootstock contained the lowest magnesium content among any of the rootstocks tested. Leaves of Marsh grapefruit trees on grapefruit rootstock contained the highest percentages of potassium. The percentages of calcium, magnesium, and potassium in the dry matter of Marsh grapefruit leaves were found to vary according to the grapefruit rootstock variety.

The values for calcium in the rootlets of Marsh grapefruit trees on various rootstocks agree with those obtained for the rootlets of Valencia orange trees on various rootstocks. Trifoliate orange and sour orange rootstocks rank high in the list for calcium percentages in the rootlets. Trifoliate orange rootstock also ranks high for the potassium values in the rootlets whereas sour orange rootstock ranks low.

For the first time data on the effect of the rootstock variety on the inorganic composition of lemon trees and fruit have been made available. In lemon peel the high calcium values were associated with sour orange and rough lemon rootstocks, and low values with sweet lemon rootstock. The high magnesium percentages in lemon peel were obtained with rough lemon, Sampson tangelo, sweet lemon, and Cleopatra mandarin rootstocks. Pomelo and tangelo rootstocks were associated with high potassium percentages in the peel of lemons and sweet lemon, rough lemon, and sour orange rootstocks with the lowest percentages.

The leaves of lemon trees contained the highest calcium percentages when the rootstock was Cleopatra mandarin and the lowest when the rootstock was sweet lemon. The pomelo and sour orange rootstocks were associated with low percentages of magnesium in the leaves and the possibility of magnesium as a limiting factor is suggested by the data presented.

Leaves of trees on pomelo, and on Sampson tangelo rootstocks, contained the highest percentages of potassium, whereas those of trees on rough lemon, sweet lemon, sour orange, and Cleopatra mandarin contained the lowest. Rootlets of lemon trees on Sampson tangelo rootstock contained the least calcium, magnesium, and potassium, whereas those of trees on African sour orange were high in calcium and low in magnesium, potassium, and total phosphorus content.

In chemical and other studies with citrus trees the determination of the rootstock variety is of considerable importance. Wide ranges in percentages were found in the inorganic composition of the various portions of citrus trees and fruits when the trees were grown on various rootstocks under approximately the same environmental conditions.

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THE CARBOHYDRATE CONSTITUENTS OF SCENEDESMUS IN RELATION TO THE ASSIMILATION OF CARBON BY PHOTOREDUCTION

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Introduction

A special type of photosynthesis which occurs in some strains of Chlorophyceae adapted by a period of anaerobiosis, was discovered and studied extensively by Gaffron (**2**). The overall chemical reaction involved in the process may be written, $CO_2 + 2H \rightarrow H_2O + (CH_2O)$. This phenomenon has been called "photoreduction" to distinguish it from ordinary photosynthesis. Certain analogies between these processes suggest similarities in their basic mechanisms. Distinguishing characteristics of photoreduction are its occurrence only under conditions of nearly complete anaerobiosis, lack of oxygen production, and absorption of hydrogen concurrently with that of carbon dioxide.

In contrast to photoreduction, the reaction of photosynthesis may be written, $CO_2 + H_2O \rightarrow (CH_2O) + O_2$. In both processes the product, represented in these equations as (CH_2O), usually is assumed to be carbohydrate Many workers have shown by measurements of gas exchange during photo synthesis that the assimilation product must have the empirical formula of carbohydrate. Aside from this, evidence regarding the identity of (CH_2O) as carbohydrate consists almost entirely in observations that carbohydrates accumulate in photosynthesizing cells.

An adequate quantitative comparison of the amount of carbon assimilated to the amount and type of carbohydrate accumulated during a period of photosynthesis by Helianthus leaves was performed with modern chemical methods by SMITH (**5**). While his analyses quantitatively accounted for the assimilated carbon chiefly as sucrose and starch, it remained an open question whether these were indeed immediate products of photosynthesis or were storage compounds transformed from one or another earlier products still unidentified. By the same logic, the work reported here is not to be construed as a search for the so-called "first product" of photoreduction, but rather it represents an attempt to determine whether or not the carbon assimilated by adapted algae in the process of photoreduction accumulates in the form of carbohydrate as was shown by Smith to be the case with photosynthesis in sunflower leaves.

Briefly stated, the results indicate that most of the assimilated carbon is not stored in the form of carbohydrate. Whether the storage material

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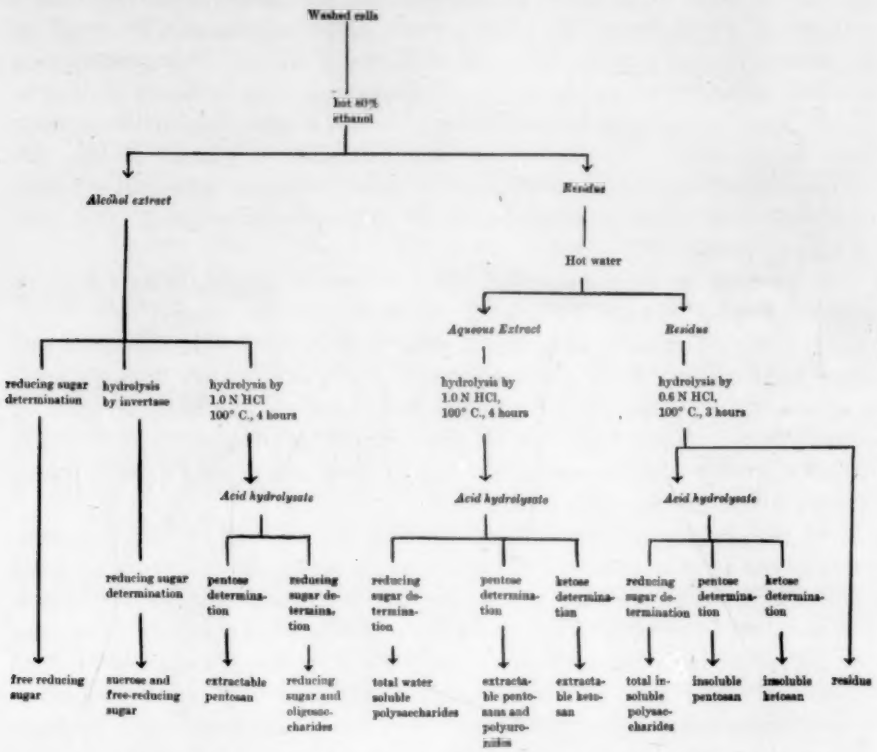
passes rapidly through a small pool of carbohydrate (which thus escapes detection) or whether the first completely reduced product of assimilation is a non-carbohydrate are questions which are as yet unanswered both for photoreduction and for photosynthesis.

Materials and methods

The experimental organism was Gaffron's D_3 strain of *Scenedesmus obliquus*. Sterile cultures were grown on inorganic liquid media in an illu-

TABLE I

FLOWSHEET OF CARBOHYDRATE ANALYSES



minated water bath. The media were aerated with CO_2 enriched air. Cultures were usually from five to seven days old at the time of harvest. After a preliminary investigation of the nature of the carbohydrate constituents of the algae, experiments were undertaken in Warburg apparatus and analyses were performed on the algae in order to detect changes in these constituents as a result of photoreduction.

Quantitative determinations of various carbohydrate fractions were accomplished by methods similar to those employed by SMITH (**5**). Table I is a flow sheet of the extraction and separation processes. Reducing sugar was

analyzed colorimetrically by the method of SOMOGYI (**6**), using NELSON'S (**3**) indicator. Pentose was determined by the method of BROWN (**1**). Ketose was determined by the method of ROE (**4**). Sucrose was determined as the difference in reducing sugar before and after hydrolysis by invertase.

Oligosaccharides other than sucrose were determined as further increase in reducing power after prolonged acid hydrolysis. Water-soluble polysaccharides were determined as reducing sugar after acid hydrolysis of a hot water extract. Water-insoluble hydrolysable polysaccharides were determined as reducing sugar after acid hydrolysis of the residue from the hot water extraction.

Results

THE NATURE OF THE CARBOHYDRATE CONSTITUENTS.—Sterile cells grown autotropically were washed free of culture medium and analyzed as indicated in table I. In the 80% ethanol extract a variable amount of free-reducing sugar was found. Usually the amount was so small that it may be suspected of being an artifact of the killing procedure attributable to hydrolysis of a non-reducing polysaccharide. Pentose determinations indicated that the free-reducing sugar was mostly hexose. Ketose assay gave results far in excess of the free-reducing sugar. Since any sucrose present would be hydrolyzed in the course of the ketose determination, sucrose was believed to be responsible for the anomaly. A gentle acid hydrolysis caused an increase in reducing sugar to a value nearly twice that obtained in ketose determinations. Hydrolysis by invertase brought about practically the same increase in reducing sugar as did mild acid hydrolysis. Table II shows a series of representative tests on extracts of different samples of algae. Apparently the free-reducing sugar was mostly aldohexose, perhaps with a small amount of ketose also present.

The quantity of sucrose present was appreciably larger than the amount of free-reducing sugar detected. In addition the alcohol extract contained a non-reducing oligosaccharide other than sucrose, for, upon vigorous acid hydrolysis, additional reducing sugar was released. A portion of this (usually between 10% and 15%) was pentose. In the water extract an appreciable amount of non-reducing polysaccharide was found. The ketose formed upon hydrolysis accounted for about one-fourth of this polysaccharide. In different samples of algae from 15% to 50% of the sugar of this hydrolysate was accounted for as pentose.

The water insoluble polysaccharides were hydrolyzed by dilute acid. The hydrolysate contained mostly aldohexose, an insignificant amount of ketose, and from 10% to 20% pentose. Over 75% of the total carbohydrate in the cell consisted of water insoluble polysaccharide. The five major carbohydrate fractions were found to account for about 20% of the dry wt. of the algae. The relative abundances of the different types can be seen in table III which shows the results of analyses on three samples of algae. Differences between samples are greater than the errors of determination and must

TABLE II

EVIDENCE FOR THE PRESENCE OF SUCROSE IN ALCOHOL EXTRACTS OF SCENEDESMUS. UNITS ARE MICROGRAMS PER ML.

A. COMPARISON OF ACID AND SUCROSE HYDROLYSES:

Test No.	Free reducing sugar before hydrolysis	Reducing sugar after dilute acid hydrolysis	Reducing sugar after invertase hydrolysis	Ratio
1	0.0	59.5	69.2	1.16
2	4.5	108.8	98.3	0.82
			Average:	0.99

B. COMPARISON OF REDUCING SUGAR PRODUCED BY HYDROLYSIS WITH TOTAL KETOSE IN HYDROLYSATE:

Test No.	Sucrose measured as reducing sugar after sucrose hydrolysis	Ketose in hydrolysate	Ratio
1	69	40	1.73
2	94	55	1.71
3	127	74	1.72
4	74	39	1.90
		Average:	1.77

be attributed to differences in the history of the cells taken for analysis; e.g., to age of culture, etc.

It is of interest that approximately 22% of the material extracted with 80% ethanol was benzene soluble. This indicates a rather large fat depot, a phenomenon which has been noted in other members of the Chlorococcales.

After the residue from the hot water extraction had been subjected to acid hydrolysis, about 35% of the original cell weight remained. More vigorous hydrolysis (more concentrated HCl for longer time) than was employed in the course of the above analyses dissolved most of this residue without, however, releasing any more reducing sugar.

CARBOHYDRATE ANALYSES BEFORE AND AFTER PHOTOREDUCTION.—After the above reported preliminary survey of the carbohydrate fractions of Scene-

TABLE III

DISTRIBUTION OF CARBOHYDRATE IN SCENEDESMUS CELLS. UNITS ARE MICROMOLES OF HYDROLYZED CARBOHYDRATE PER GRAM DRY WEIGHT AND PER CENT OF TOTAL DRY WEIGHT OF CELLS

CARBOHYDRATE FRACTION	Sample 1		Sample 2		Sample 3	
	$\mu Mg.^{-1}$	%	$\mu Mg.^{-1}$	%	$\mu Mg.^{-1}$	%
Free-reducing sugar	4	0.1	3	0.1	3	0.1
Sucrose	44	0.8	200	3.4	89	1.6
Oligosaccharide (other than sucrose)	74	1.2	53	0.9	42	0.7
Water-soluble polysaccharide	40	0.6	13	0.2	6	0.1
Insoluble polysaccharide	1020	16.9	9325	15.1	9440	15.3
Total dry weight %	.....	19.6	.....	19.7	.....	17.8

desmus, a photoreduction experiment was run for the purpose of determining whether any of these fractions were significantly increased.

A 24-ml. aliquot of an algal suspension, containing 4.8 volume per cent cells, was distributed among four Warburg vessels. The cells were adapted overnight in the dark in an atmosphere consisting of a mixture of 4% CO_2 in hydrogen. Upon illumination the cells commenced photoreduction which was permitted to continue for 10 hours, during which time the rates were measured intermittently and additional $CO_2H_2 = 1:2$ mixture occasionally was added to the gas phase of the vessels to replace that absorbed by the algae. At the end of this time the cells were killed in hot 80% ethanol and were analyzed in parallel with a sample of the original suspension which had not been illuminated before killing.

The product of assimilation could not be accounted for quantitatively but the analyses indicated an increase in total carbohydrate attributable to photoreduction, especially in the polysaccharide fraction. However, the results were not conclusive since differences were not far greater than the analytical errors. Assimilation computed from manometric data amounted to the equivalent of about 3.5 mg. carbohydrate. This amount was not sufficient to insure clear-cut results when attempting to account quantitatively for increases among the carbohydrate fractions.

TABLE IV

CARBOHYDRATE ANALYSES OF SAMPLES OF ALGAE BEFORE AND AFTER PHOTOREDUCTION. EACH SAMPLE CONTAINED 423 MG. DRY WT. RESULTS OF CARBOHYDRATE ANALYSES ARE COMPUTED IN MG. RESIDUE WEIGHTS ALSO ARE INCLUDED

Fraction	Control (before photoreduction)	After 30 hours photoreduction
Free-reducing sugar	0.2	0.2
Sucrose	14.5	6.8
Oligosaccharide (other than sucrose)	3.7	2.9
Water-soluble polysaccharide	0.9	0.4
Insoluble polysaccharide	64.0	64.7
Non-hydrolysable residue	148.1	147.1

Accordingly, a longer experiment was performed on 34 ml. suspension containing 1.69 ml. cells distributed among six Warburg vessels. The time of illumination was 30 hours during which assimilation was equivalent to about 18 mg. carbohydrate (39 ml. gas uptake at $CO_2/H_2 = 1/2$). Instead of such an easily detectable increase, small decreases generally occurred. Table IV shows a comparison of results of analyses on illuminated and control cells. It is apparent that no single carbohydrate fraction could be considered a storage depot for the assimilated carbon.

Discussion

In spite of Gaffron's demonstration that the assimilatory quotient of photoreduction ($\Delta H_2/\Delta CO_2$) is equal to 2 and that the comparable quotient

for photosynthesis ($\Delta O_2/\Delta CO_2$) has been found by numerous workers to be equal to unity, it is significant that autotropically grown Scenedesmus have an elementary composition approximately corresponding to $C_6H_{11}O_3$ per atom of nitrogen. Clearly such material cannot be synthesized by processes which form an assimilatory product with the empirical formula, CH_2O as necessitated by the respective quotients both of photoreduction and of photosynthesis. In this connection, it is of importance to note that prolonged assimilation at light intensities which are saturating for photosynthesis eventually lead to inhibition (solarization). The cell appears incapable of infinite storage of photosynthate without opportunity for transforming it into other products of its own average composition, which it must do by dark respiration with an R.Q. greater than unity.

We know from experiments still in progress in this laboratory that tracer carbon, assimilated by Scenedesmus in the course of photosynthesis or of photoreduction, distributes itself generally throughout the cell in a matter of a few minutes. For example, we have mentioned previously the large fat content of these algae (which is mainly responsible for the fact that the cell substance is considerably more reduced than carbohydrate). This fat depot rapidly accumulates an appreciable fraction of the C^*O_2 fed to the cell. In view of these recent findings, it is not surprising that, in the experiments reported here which were performed without the aid of tracer carbon, it proved impossible to detect the stored photosynthate as carbohydrate; too large a fraction of the assimilated carbon accumulated as non-carbohydrate during the long period of the experiments. That shorter periods of illumination would have increased the amount of carbohydrate accumulated relative to the total assimilation cannot be ruled out on the basis of the data presented here. However, more recent experiments with tracer carbon demonstrated conclusively that at no time is the assimilatory product chiefly carbohydrate. These latter findings will be published shortly (**7**).

Summary

The carbohydrate fractions of the green alga, Scenedesmus, were investigated quantitatively. Analyses made after an extended period of photoreduction showed no marked increase in any of the carbohydrate fractions over corresponding values for control cells. Therefore, the assimilation of carbon dioxide in the course of prolonged photoreduction must lead to an increase in storage products which are not carbohydrates. The results clearly indicate the need for experiments with tracer-carbon to ascertain the fate of the assimilation product.

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THE INFLUENCE OF CERTAIN ROOTSTOCKS AND SCIONS ON THE ASCORBIC ACID CONTENT OF POTATO TUBERS

W. C. Kelly and G. F. Somers

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During the course of a genetic study of the ascorbic acid (vitamin C) content of potatoes, it was found that large differences in ascorbic acid existed between varieties. Is this variation in ascorbic acid content due to the genetic constitution of the aerial portion or to the genetic constitution of the underground portion of the potato plant? To study this question an experiment was designed in which plants were produced with aerial and underground portions of different genetic constitution. This was done by grafting scions from varieties which produce tubers with a low ascorbic acid content on rootstocks of varieties which produce tubers with a high ascorbic acid content. The reverse combination was also made. In addition, scions of each variety were grafted on their own rootstocks. Thus, it was possible to separate the effect of the genetic constitution of underground and of aerial portions of potato plants on the ascorbic acid content of the tubers.

Procedure

Six varieties of potatoes were selected for the experiment, three producing tubers having a high ascorbic acid content and three with a low ascorbic acid content. The varieties producing high ascorbic acid values were: I.V–2 1965 (Smooth Rural × Katahdin) × Katahdin; III.V–2 2353 (Red McClure × Katahdin), and V.3308 Rural New Yorker × Katahdin. Those with low ascorbic acid values were: II.V–3 4497 (Russet Rural × Katahdin) × (Russet Rural × Green Mountain); IV.V–3 828 Black Congo × Ostragis, and VI.V–3 4329 (Smooth Rural × Katahdin) × Ostragis. In the following discussion these varieties will be referred to by number only (I, II, etc.). The seed potatoes for this experiment were furnished by Dr. J. R. Livermore of the Department of Plant Breeding, Cornell University.

Cured two-ounce seed-pieces were planted June 14, 1946, in two-gallon jars filled with quartz sand. The crocks were supplied three times weekly with 1½ liters per jar of Hoagland's (1) solution No. 1, and distilled water was applied as needed by the plants. When the plants were about six inches tall (July 12), grafting was begun. The varieties were paired and ten plants of each variety within a pair were reciprocally grafted. In addition ten plants of each variety were grafted on their own rootstocks; also, ten rootstocks of variety I were grafted with Bonny Best tomato scions.

The grafting was performed by means of a modified cleft graft. The stem of the rootstock plant was severed at a right angle to the axis about two inches from the level of the sand. The stump was split longitudinally and a scion about the same diameter as the stock was trimmed to a wedge

and inserted in the split. The union was wound with raffia and covered with grafting wax. All leaves were removed except the partially expanded apical leaves. The completed grafts were shaded with cloth which was kept wet throughout the day by means of an automatic lawn sprinkler. Each treatment was replicated ten times, and whenever possible all plants in a block were grafted the same day. The plants were harvested September twenty-fourth. The tops were severed at the surface of the sand, weighed for fresh weight, dried for 48 hours at 70° C. in a forced draft oven, and weighed for dry weight. The tubers were washed free of sand, counted, weighed, and placed at 40° F. until analysis the following day.

Ascorbic acid was determined by the method described by NELSON and SOMERS (**2**). Usually two tubers weighing more than 30 grams were sampled from each plot. In a few cases only one tuber was sampled or smaller tubers were used. Unpublished data of the authors indicate no influence of size of tuber on ascorbic acid content. The tubers were sampled by cutting a median longitudinal slice weighing about 20 grams or by analyzing the whole tuber in the cases where the tubers did not weigh more than 20 grams. It was found that the ascorbic acid content of leaves increased from the base to the apex of the plant. Hence for the leaflet analysis, three grams of leaflets were stripped from the petioles of the middle leaves of each plant. Fully expanded leaves showing no signs of senescence were selected for analysis. Standard errors were calculated for each mean, and significance of the difference between two means was determined by calculation of Student's "t." Odds greater than 19:1 were considered significant.

Data and discussion

In most cases, the scions recovered from wilting in two or three days, and began to grow within about ten days. However, the reciprocal grafts between varieties V and VI proved incompatible. The plants recovered from wilting and began to grow as did the other varieties, but after four or five new leaves had expanded, the plants became extremely chlorotic and died within two or three weeks. None of these grafts survived. The number of plants surviving for the other combinations is presented in table I. The low survival of the reciprocal grafts between varieties I and II was probably due to the difficulty of obtaining stocks and scions of the same size.

There were no significant differences between the grafted combinations of varieties I and II in regard to number and weight of tubers and fresh and dry weight of tops (tables I and II). The ascorbic acid content of the leaflets of II was significantly higher than I. This relationship was not altered by the nature of the rootstock. The ascorbic acid content of the tubers of I was significantly higher than II, regardless of the nature of the scion. Thus, II had a higher ascorbic acid content in the leaflets than I, but had a lower ascorbic acid content in the tubers. Grafting potato rootstocks with scions producing leaflets with different ascorbic acid content did not alter the ascorbic acid content of the tubers.

TABLE I

THE EFFECT OF VARIOUS GRAFT COMBINATIONS ON THE ASCORBIC ACID CONTENT OF LEAFLETS AND GROWTH OF THE AERIAL PORTION OF POTATO PLANTS

Treatment		Leaflets Ascorbic acid		Fresh Weight per plant		Dry Weight per plant		No. of plants surviving
Scion — Stock		Mean ± S.E. (*mg./100gm.*)		Mean ± S.E. (*grams*)		Mean ± S.E. (*grams*)		
I	I	130.5	5.72	85.9	17.3	9.1	1.58	8
II	I	153.2	6.79	102.2	15.5	8.6	2.35	4
II	II	168.3	6.61	102.9	18.4	9.2	1.92	10
I	II	117.8	11.60	86.5	11.0	7.4	1.08	5
Tomato	I	.....		187.3	18.0	14.1	1.96	7
Tomato	Tomato	.....		249.1	20.7	25.9	2.70	10
III	III	102.9	4.02	146.3	19.5	14.6	2.25	10
IV	III	114.8	11.04	58.3	6.3	5.4	2.17	9
IV	IV	115.4	6.02	171.5	19.1	17.7	2.16	10
III	IV	108.0	7.22	137.3	14.5	13.5	1.68	8

The ascorbic acid content of the leaflets of varieties III and IV did not differ significantly, but the tubers of III had a significantly higher ascorbic acid content than the tubers of IV. There was, also, a significant difference between the yields of the various graft combinations of III and IV. Variety IV grafted on rootstocks of III resulted in highly significant reduction in yield of tops and tubers when compared to the other combinations of these varieties. Even though the yield of tubers of III was reduced when grafted with scions of IV, there was no change in the ascorbic acid content of the tubers of III. Thus, the ascorbic acid content of the tubers was determined by the nature of the rootstock without regard to the nature of the scion or the effect of the scion on the yield of tubers. A further indica-

TABLE II

THE EFFECT OF VARIOUS GRAFT COMBINATIONS ON THE WEIGHT, NUMBER, AND ASCORBIC ACID CONTENT OF POTATO TUBERS

Treatment		Ascorbic acid		Fresh Weight per plant		Number of tubers per plant	
Scion — Stock		Mean ± S.E. (*mg./100 gm.*)		Mean ± S.E. (*grams*)		Mean ± S.E.	
I	I	42.92	3.13 (15)*	196.3	14.7	6.50	1.31
II	I	40.62	2.93 (8)	290.5	71.9	6.00	1.15
II	II	25.85	1.32 (19)	210.5	28.5	4.80	0.95
I	II	27.75	1.26 (8)	298.8	52.5	6.60	1.81
Tomato	I	39.44	3.06 (12)	84.7	14.1	5.29	0.68
III	III	39.70	1.05 (19)	232.1	43.1	7.30	1.34
IV	III	38.93	1.37 (15)	110.4	23.6	3.44	0.95
IV	IV	29.84	0.83 (19)	304.3	32.4	10.80	1.99
III	IV	29.29	0.81 (16)	225.1	26.5	7.38	1.13

* Numbers in parentheses indicate the number of tubers analyzed for ascorbic acid.

tion of the importance of the rootstock in determining the ascorbic acid content of potato tubers occurred where potato rootstocks of variety I were grafted with tomato scions. There was a significant reduction in yield of tubers, but the ascorbic acid content of the tubers was not significantly changed. Thus, the ascorbic acid content of potato tubers was not affected by the nature of the aerial portion of the plant even when the aerial portion was a different species of plant.

The results of this experiment indicate that the ascorbic acid content of potato tubers was not influenced by the nature of the scion, but was determined by the nature of the rootstock. This relationship was not affected even if the scions had leaflets of different ascorbic acid contents, reduced the yield of tubers, or were of different species of plants. It should not be assumed that the aerial portion of the potato plant is entirely without influence on the ascorbic acid content of the tubers. SMITH and GILLIES (3) reported that the ascorbic acid content of potato tubers decreased as soon as the tops died. This observation has been confirmed by unpublished data (Kelly and Somers) of this laboratory. Apparently living leaves and stems are essential for maintenance of a certain level of ascorbic acid in the tubers, which is determined by the nature of the underground portion of the plant.

Differences in ascorbic acid content of the leaflets or in yield of tops did not affect the ascorbic acid content of potato tubers. In one pair of varieties, the one with the lower ascorbic acid content of the leaflets had tubers with the higher ascorbic acid content. It follows that in breeding potatoes for high ascorbic acid content, little or no emphasis should be placed on the ascorbic acid content of the aerial portion of potato plants.

Summary

Reciprocal grafts were made between potato varieties having tubers with different ascorbic acid contents. In addition, potato rootstocks were grafted with tomato scions. Although differences in weight of tops and tubers and ascorbic acid content of leaflets resulted, the ascorbic acid content of the tubers was not affected by the nature of the scion. The ascorbic acid content of potato tubers was regulated by the genetic constitution of the underground portion of potato plants regardless of the genetic constitution of the aerial portion of the plant.

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THE EFFECT OF THREE FACTORIAL LEVELS OF NITROGEN AND PHOSPHORUS ON THE GROWTH AND COMPOSITION OF *CINCHONA LEDGERIANA*

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(WITH ONE FIGURE)

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Most of the cultural trials and experiments with cinchona prior to 1900 were recorded as observations. With the possible exception of the work by COWGILL (**1**) the published information with reference to the effects of fertilizer applications on growth and alkaloid content of cinchona deal mainly with established trees (**3, 4, 8, 9, 11**). Although it is generally conceded that the first few months are the most critical period in the life of the cinchona plant, little or no experimental data are available as to the nutritional requirements of young seedlings.

Nitrogen and phosphorus are two essential mineral elements that are usually deficient in tropical soils. In view of this fact an experiment was initiated to obtain information on the effects of three levels of each of those elements and their inter-relationship on the growth and composition of young cinchona seedlings.

Materials and methods

Seedlings of *Cinchona ledgeriana* selected for uniformity were transplanted in July 1946 to 5-gallon crocks filled with medium coarse silica sand. Five seedlings were planted to each crock. One replication which consisted of nine crocks was placed in each of three air-conditioned greenhouse chambers described elsewhere (**10**). The temperature in all chambers was maintained at 75° F. during the day and 65° F. at night. In a previous experiment (**10**), this temperature was found to be the most favorable for growth of *C. ledgeriana*. Relative humidity was maintained as high as possible by continually spraying the floors with water. During the middle of the day relative humidity usually dropped to 60 or 70% but most of the time it ranged between 80 and 95%.

During July and August the plants in all crocks were supplied with Hoagland and Arnon's complete nutrient solution (**5**) diluted to ¼ strength and adjusted to pH 5.5. One gallon of solution was applied to each crock by pouring on the surface of the sand three or four times a week. A three-quarter-inch hole plugged with glass wool at the bottom of each crock provided adequate drainage.

In September, two or three of the weakest plants in each pot were removed and the differential nutrient solution treatments started. These consisted of three levels of nitrogen and three levels of phosphorus in factorial combination. The low, medium, and high levels of nitrogen were supplied at

3, 18, and 81 p.p.m. and the low, medium, and high levels of phosphorus were supplied at 0, 5, and 25 p.p.m. respectively. The other essential mineral elements were maintained at equal levels in all solutions. The composition of the nine factorial nutrient solutions are given in table I. Each nutrient solution was made up in 50-gallon drums, adjusted to pH 5.5 and applied as described previously. The position of the crocks within each chamber or replication was randomized.

After 5 months of treatment the plants were harvested and growth data which included height, fresh and dry weight, and top-root ratio were obtained. The roots and stems were analyzed for total alkaloid and quinine sulfate content by the method described by LOUSTALOT and PAGAN (**6**). The leaves were analyzed for nitrogen, phosphorus, potassium, calcium, and magnesium by the methods outlined by DROSDOFF and PAINTER (**2**).

Results

EFFECT OF TREATMENTS ON GROWTH

The first differences due to treatment were evident in the plants of low nitrogen treatment. About one month after the treatments were started the leaves of those plants were lighter green in color than those of plants in the higher nitrogen treatments and there was a definite check in growth. The chlorosis became more intensified with time. The higher levels of phosphorus in the N_1 group seemed to intensify the effects of low nitrogen. The plants in those treatments appeared more chlorotic than those of the N_1P_1, and, in addition, had considerable red coloration in the young leaves, stems, and petioles. This was more pronounced in the N_1P_3 plants than in the N_1P_2 plants. The leaves of plants in the N_2 series in general were intermediate in color between those of the N_1 and N_2 groups. The effect of high phosphorus in the N_2 group was similar to that in the N_1 series but to a lesser degree. The plants in the high phosphorus treatment of the N_2 level were considerably less green and had more red coloration than those of the P_1 and P_2 plants at the same nitrogen level.

The seedlings grown at the high nitrogen level (N_3) had large dark-green leaves and thick stems and there was no reddening as in the N_1 and N_2 groups regardless of phosphorus level. The plants in the low phosphorus treatment had narrow and darker colored leaves than those of plants in the P_2 and P_3 levels. However, there were no other clear-cut symptoms that could be attributed to phosphorus deficiency.

The growth data presented in table II show that the low level of nitrogen had a profound depressing effect on the growth of plants in this treatment. The height and fresh and dry weights of seedlings receiving the low level of nitrogen were significantly lower than those of plants grown at the medium and high levels of nitrogen, but there was no statistically significant difference between the growth of the N_2 and N_3 plants.

The high level of phosphorus had a depressing effect on growth in the low nitrogen plants and to a lesser extent in the medium nitrogen group. In the

TABLE I

COMPOSITION OF NITROGEN-PHOSPHORUS NUTRIENT SOLUTIONS*

TREATMENT NO.	NITROGEN AND PHOSPHORUS LEVELS		NITROGEN	PHOSPHORUS
			p.p.m.	*p.p.m.*
1		P_1	3	0
2	N_1	P_2	3	5
3		P_3	3	25
4		P_1	18	0
5	N_2	P_2	18	5
6		P_3	18	25
7		P_1	81	0
8	N_3	P_2	81	5
9		P_3	81	25

* The concentration of other essential elements in all solutions were as follows: K—40 p.p.m.; Ca—232 p.p.m.; Mg—48 p.p.m.; S—48 p.p.m.; Fe—3 p.p.m.; Mn—0.5 p.p.m.; B—0.5 p.p.m.; Zn—0.5 p.p.m.; and Cu—0.1 p.p.m.

high nitrogen series the reverse was true. The least growth of the N_3 plants was made when phosphorus was low and vice versa. These results are in agreement with those of COWGILL (1). He conducted an N, P, K, factorial experiment with *Cinchona ledgeriana* in sand culture and also concluded that phosphorus is required in relatively small amounts by young cinchona seedlings and that growth may be limited under certain conditions if the phosphorus concentration is too high. He states further that although the relative requirement for phosphorus is low, it must be available in the proper proportion to nitrogen and possibly potassium.

The percentage of dry matter in the low nitrogen plants was significantly higher than that in the medium and high nitrogen plants. This is not surprising since the growth of the N_1 plants was retarded as a result of nitrogen deficiency. The top-root ratio of the low and medium nitrogen seedlings

TABLE II

GROWTH OF *Cinchona ledgeriana* UNDER THREE FACTORIAL LEVELS OF NITROGEN AND PHOSPHORUS

TREATMENT NO.	NITROGEN AND PHOSPHORUS LEVELS		AVERAGE HEIGHT*	AVERAGE FRESH WEIGHT	AVERAGE DRY WEIGHT	DRY MATTER	TOP-ROOT RATIO
			cm.	*gm.*	*gm.*	%	
1		P_1	48.4	52.1	11.6	25.4	1.04
2	N_1	P_2	53.7	66.5	15.7	23.6	1.00
3		P_3	45.8	35.0	8.4	24.0	1.03
4		P_1	71.4	120.0	26.5	22.1	1.00
5	N_2	P_2	75.1	125.3	26.7	21.3	1.07
6		P_3	70.1	104.0	23.9	22.9	0.83
7		P_1	61.1	83.0	18.3	22.0	1.37
8	N_3	P_2	85.1	128.3	26.9	21.0	2.30
9		P_3	76.2	136.8	28.5	20.8	1.60

* Each figure is an average of 6 to 9 plants.

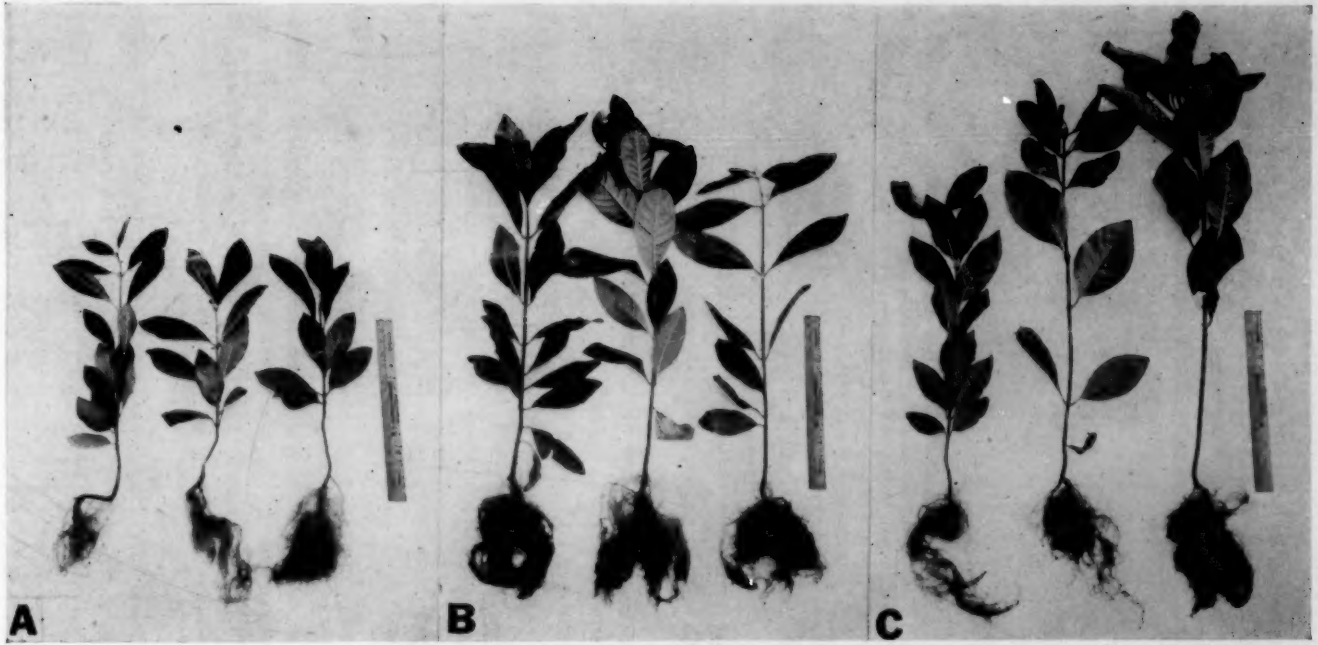

Fig. 1.—Left to right: Typical *Cinchona ledgeriana* seedlings grown at three factorial levels of nitrogen and phosphorus. (A) Low nitrogen series, (B) medium nitrogen series, and (C) high nitrogen series. In each series from left to right are the low, medium, and high phosphorus plants. Note depressing effect on growth of low phosphorus only in high nitrogen series.

was significantly lower than that of the plants in the high nitrogen treatment. Photographs of representative plants from all treatments are shown in figure 1.

Effect of Treatments on Total Alkaloid and Quinine Sulfate Content

The total alkaloid and quinine sulfate content of the roots and stems are given in table III. In general the roots and stems of plants grown at the high nitrogen level contained significantly higher amounts of total alkaloid and quinine sulfate than did those of plants in the medium and low nitrogen treatment. There was no statistically significant difference in the amounts of these constituents between roots and stems of the N_1 and N_2 plants. However, there was a tendency for them to be higher in tissues of the medium nitrogen plants than in those of the low nitrogen seedlings. There was no consistent effect of phosphorus on the quinine sulfate content of the plants in the various treatments, but there was a definite tendency for the total alkaloids to be higher in the plants grown at a high phosphorus level than in those grown at a low phosphorus level.

TABLE III

TOTAL ALKALOID AND QUININE SULFATE CONTENT OF *Cinchona ledgeriana* SEEDLINGS GROWN UNDER THREE FACTORIAL LEVELS OF NITROGEN AND PHOSPHORUS*

Treatment No.	Nitrogen and phosphorus levels		Total alkaloids		Quinine sulfate	
			Stems	Roots	Stems	Roots
			%	%	%	%
1		P_1	2.93	3.05	0.46	0.81
2	N_1	P_2	2.99	3.57	0.37	1.06
3		P_3	3.91	3.01	0.43	1.17
4		P_1	3.65	3.91	0.50	1.21
5	N_2	P_2	3.22	3.64	0.42	1.22
6		P_3	3.76	4.28	0.46	1.00
7		P_1	3.79	3.72	0.68	1.40
8	N_3	P_2	4.22	4.25	0.69	1.27
9		P_3	4.23	4.43	0.54	1.43

* The authors are indebted to Caleb Pagán for making these analyses.

Effects of Treatments on Mineral Composition of Leaves

It is evident from the data presented in table IV that the treatments had a marked effect on the mineral composition of the leaves. As would be expected the nitrogen content of the seedlings was directly correlated with the level of nitrogen at which they were grown, i.e., the low nitrogen plants contained the least amounts of the element, the high nitrogen had the highest, and the medium nitrogen were intermediate in nitrogen content. There was a consistent effect of the phosphorus levels on the nitrogen content of the leaves. At all nitrogen levels, the high phosphorus plants contained less nitrogen than did the low phosphorus plants. These data indicate that applications of phosphorus should not be made to young

cinchona trees when nitrogen supply tends to be low because the addition of phosphorus might intensify the nitrogen deficiency. This was evident from the retarded growth of the plants in the high phosphorus low nitrogen treatment.

The potassium content of the leaves was not appreciably affected by the nitrogen levels, although there was a tendency for potassium to be somewhat higher in leaves of the N_3 plants than in those of the N_1 and N_2 seedlings. The potassium content of low and medium nitrogen plants at the high phosphorus level was considerably less than similar plants grown in low phosphorus treatments. At the N_3 level phosphorus had no appreciable effect on the potassium content of the leaves.

The calcium content of the leaves from plants grown in high nitrogen solution was consistently higher than that of leaves from medium and low

TABLE IV

MINERAL COMPOSITION OF LEAVES OF *Cinchona ledgeriana* GROWN UNDER THREE FACTORIAL LEVELS OF NITROGEN AND PHOSPHORUS

TREATMENT NO.	NITROGEN AND PHOSPHORUS LEVELS		NITROGEN	PHOSPHORUS	POTASSIUM	CALCIUM	MAGNESIUM
			%	%	%	%	%
1		P_1	1.28	0.24	1.14	0.43	0.29
2	N_1	P_2	1.13	0.28	1.14	0.53	0.26
3		P_3	1.10	0.49	0.82	0.67	0.21
4		P_1	1.70	0.20	1.21	0.45	0.28
5	N_2	P_2	1.45	0.25	1.07	0.57	0.24
6		P_3	1.28	0.37	0.83	0.63	0.22
7		P_1	2.45	0.20	1.13	0.75	0.28
8	N_3	P_2	2.30	0.21	1.15	0.72	0.27
9		P_3	2.19	0.24	1.19	0.70	0.25

nitrogen plants. There was no marked effect of phosphorus on calcium content of the N_3 level but at the N_2 and N_1 levels of nitrogen the amount of calcium in the leaves was directly correlated with the level of phosphorus.

The nitrogen levels had no appreciable effect on the magnesium content of the leaves. However, at all nitrogn levels there was a small but consistent effect of phosphorus on the magnesium content. Magnesium was slightly lower at the high phosphorus level.

Discussion

The results of this experiment, insofar as they are applicable to field conditions, indicate that young cinchona trees are particularly sensitive to the fertilizer balance. Careful consideration should be given to all kinds and amounts of fertilizer and the ratios of the fertilizer elements applied. From the data in this paper this is especially true for nitrogen and phosphorus. Nitrogen supply to cinchona seedlings is important both from the standpoint of better growth and higher content of quinine and other alkaloids. Of special interest is the fact that supplying 18 p.p.m. of nitrogen to the seed-

lings resulted in almost as good growth as 81 p.p.m., indicating that nitrogen concentrations much above this level are not warranted.

Of fundamental interest is the effect of nitrogen and phosphorus levels on mineral composition of the leaves. Leaves of plants supplied a high level of nitrogen had a fairly uniform composition regardless of phosphorus level with the exception of the nitrogen content which varied inversely with the phosphorus supply. However, in plants where nitrogen level was medium or low the phosphorus level had a definite effect on the mineral composition of the leaves. In these plants the percentages of calcium and phosphorus increased as the phosphorus supply increased but the amounts of nitrogen, potassium and magnesium decreased. These results are additional evidence in support of the nutrient-element balance concept proposed by SHEAR, CRANE, and MYERS (**7**). These authors state "as any element decreases or increases substantially from its concentration of optimum intensity, the maximum growth possible within the new limits of supply of that element can result only when the concentration of all elements have been brought into balance at the new level of intensity determined by that element." Increasing the level of one element (as phosphorus, in the present experiment) while maintaining another element (nitrogen) at a deficient or low level resulted in an unbalanced condition in regard to other elements even though they were available in all treatments in equal concentration.

Summary

1. *Cinchona ledgeriana* seedlings were grown in sand culture at three levels of nitrogen (3, 18, and 81 p.p.m.) and three levels of phosphorus (0, 5, and 25 p.p.m.) in factorial combination.

2. The low level of nitrogen had a marked depressing effect on the growth of the seedlings, but there was no statistically significant difference in the growth of plants receiving 18 and 81 p.p.m. of nitrogen.

3. The high level of phosphorus greatly depressed growth of plants with the low nitrogen supply and to a lesser extent those supplied with a medium nitrogen level. On the other hand growth of the high nitrogen plants was directly correlated with phosphorus level.

4. Roots and stems of plants grown at the high nitrogen level contained higher amounts of total alkaloid and quinine sulfate than did those of plants grown at the lower nitrogen levels.

5. There was no consistent effect of phosphorus on the quinine content of the plants in the various treatments but there was a tendency for total alkaloids to be higher in plants with a high phosphorus level.

6. The nitrogen content of the leaves varied directly with the nitrogen level at which the plants were grown. At all nitrogen levels the nitrogen content of the leaves was inversely correlated with the phosphorus supply. Thus, at the low nitrogen level the high phosphorus concentration accentuated nitrogen deficiency.

7. The leaves of plants grown in the low and medium nitrogen levels

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The potassium content of the leaves was not appreciably affected by the nitrogen levels, although there was a tendency for potassium to be somewhat higher in leaves of the N_3 plants than in those of the N_1 and N_2 seedlings. The potassium content of low and medium nitrogen plants at the high phosphorus level was considerably less than similar plants grown in low phosphorus treatments. At the N_3 level phosphorus had no appreciable effect on the potassium content of the leaves.

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TABLE IV

MINERAL COMPOSITION OF LEAVES OF *Cinchona ledgeriana* GROWN UNDER THREE FACTORIAL LEVELS OF NITROGEN AND PHOSPHORUS

TREATMENT NO.	NITROGEN AND PHOSPHORUS LEVELS		NITROGEN	PHOSPHORUS	POTASSIUM	CALCIUM	MAGNESIUM
			%	%	%	%	%
1		P_1	1.28	0.24	1.14	0.43	0.29
2	N_1	P_2	1.13	0.28	1.14	0.53	0.26
3		P_3	1.10	0.49	0.82	0.67	0.21
4		P_1	1.70	0.20	1.21	0.45	0.28
5	N_2	P_2	1.45	0.25	1.07	0.57	0.24
6		P_3	1.28	0.37	0.83	0.63	0.22
7		P_1	2.45	0.20	1.13	0.75	0.28
8	N_3	P_2	2.30	0.21	1.15	0.72	0.27
9		P_3	2.19	0.24	1.19	0.70	0.25

nitrogen plants. There was no marked effect of phosphorus on calcium content of the N_3 level but at the N_2 and N_1 levels of nitrogen the amount of calcium in the leaves was directly correlated with the level of phosphorus.

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The results of this experiment, insofar as they are applicable to field conditions, indicate that young cinchona trees are particularly sensitive to the fertilizer balance. Careful consideration should be given to all kinds and amounts of fertilizer and the ratios of the fertilizer elements applied. From the data in this paper this is especially true for nitrogen and phosphorus. Nitrogen supply to cinchona seedlings is important both from the standpoint of better growth and higher content of quinine and other alkaloids. Of special interest is the fact that supplying 18 p.p.m. of nitrogen to the seed-

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6. The nitrogen content of the leaves varied directly with the nitrogen level at which the plants were grown. At all nitrogen levels the nitrogen content of the leaves was inversely correlated with the phosphorus supply. Thus, at the low nitrogen level the high phosphorus concentration accentuated nitrogen deficiency.

7. The leaves of plants grown in the low and medium nitrogen levels

contained increased percentages of calcium and phosphorus as the phosphorus supply increased but the amounts of nitrogen, potassium, and magnesium, in these plants decreased.

8. The results of this experiment indicate that growth of young cinchona trees may be limited under certain conditions if the phosphorus concentration is too high. They also indicate that the phosphorus requirement of cinchona is relatively low and that for optimum growth the phosphorus must be available in the proper proportion to nitrogen and possibly other mineral elements.

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PHOSPHORUS TURNOVER AND PHOTOSYNTHESIS[1]

S. ARONOFF AND M. CALVIN

WITH ONE FIGURE

Received November 21, 1947

The participation of phosphorus in biological oxidation-reduction reactions of the type found in glycolysis: ADP + PO_4H^- + 3-phosphoglyceraldehyde + DPN^+ = 3-phosphoglycerate$^-$ + $2H^+$ + DPNH + ATP, has suggested theories in which similar reactions are proposed for photosynthesis (LIPMANN **6**, RUBEN **8**, EMERSON, STAUFFER, and UMBREIT **2**). In these theories the reducing power of photosynthesis is utilized not only for reduction of carbon dioxide but also, by means of coupled oxidations, for the generation of high-energy phosphate bonds, or, in the last reference, directly for the generation of high-energy phosphate. Since in these theories acyl phosphate is formed from inorganic phosphate, the concepts are amenable to test without isolation of particular intermediates, by means of radioactive phosphorus. It would be expected that the rate of conversion of inorganic phosphate to organic phosphate would be greater in light than in the dark. We have investigated this possibility under a variety of conditions. The following experiments are reported:

a) WITH GRANA. The use of grana, which are capable of accomplishing the photochemical reduction of a variety of substrates, e.g. of quinones (WARBURG **9**, FAN *et al.* **3**, ARONOFF **1**), permits a test of the theory of EMERSON *et al.* It is to be expected that the direct utilization of light should result in the formation of organic phosphate.

b) WITH LEAVES. There is some evidence to indicate that the light reaction involving the fixed carbon dioxide occurs within the chloroplast (FRENKEL, **4**). This suggests that the chloroplasts, isolated from lighted and darkened halves of a leaf will show differences in rate of formation of organic phosphate.

c) WITH ALGAE. Diverse aspects were investigated; light vs. dark, the presence and absence of carbon dioxide and of oxygen.

Method

The P* obtained as carrier-free $HPO_4^=$ had a specific activity of approximately 35 $\mu c/\gamma$, the concentration of the P* unfortunately varying from sample to sample and commonly being as high as 10 γ/ml. Experimental total counts of P* were of the order of $10^6 \rightarrow 10^7$, involving aliquots of $0.1 \rightarrow 0.2$ ml. of the P* solution. The amount of absorption of P* into e.g. 100 c.mm. algae during a normal run of ca. 15 minutes is of the order of

[1] Contribution from the Radiation Laboratory and the Department of Chemistry of the University of California, Berkeley.

0.1 → 5% depending upon particular conditions. All experiments with algae were performed with the usual manometric equipment, P^* being tipped in after equilibration. The determination of the activity in the algae was accomplished, after quantitative removal from the reaction vessels, by centrifugation (complete within 2.5′, 2°–3° C.), and washing twice with cold distilled water, and finally being made to volume 2, 5 or 10 ml. (depending on activity of P^* or number of cells) with cold M/10 KH_2PO_4. An aliquot of this suspension was pipetted onto a counting plate, made by glueing filter paper onto an aluminum disc with rubber cement. (In preparation

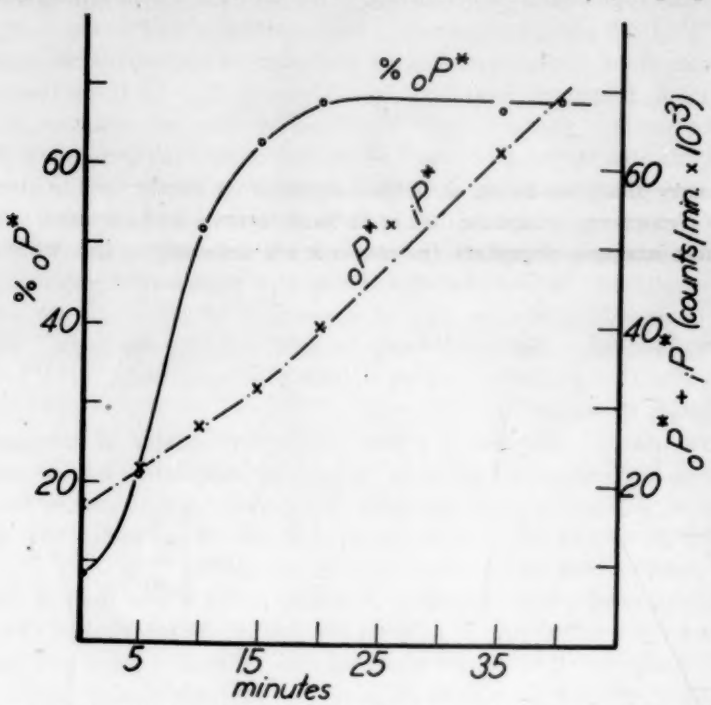

FIG. 1. The rate of conversion of inorganic to organic phosphorus by aerobic algae (for explanation see text).

the use of the rubber cement should not be minimized, and pressure should be applied during drying.) With our activities and cell numbers, counts on a plate usually were of the order of 100 → 1000/minute.

The method is based on the observation that two washings of cells with distilled water remove external radio-phosphate completely (less than 0.01%) and further washings do not remove additional phosphate from the cells. The suspension of the cells in M/10 $H_2PO_4^-$, for a period of 8–10 minutes, results in a semi-quantitative exchange between the relatively concentrated external inactive phosphate and the intracellular inorganic radio-phosphate. Different cultures appeared to require varying pH's, up to 6.5 for quantitative removal of $_iP^*$ (inorganic radio-phosphate). Centrifuga-

tion and resuspension of the cells in $H_2PO_4^-$ does not cause appreciable additional removal of phosphate. However, periods longer than the 8–10 minutes diminish the apparent organic P* ($_oP^*$) since there is continual interchange ($_oP^* \leftrightarrows {}_iP^*$) between external P ($_eP$) and $_iP^*$. The extension of the suspension period e.g. from 10′ → 30′ results in an apparent diminution of $_oP^*$ from 80% (at equilibrium, $_oP/{}_iP = 4$) → 65% (fig. 1). This is complicated by the $_iP \rightarrow {}_oP$ change, but the use of cold solutions diminishes the rate of these processes. Results within a particular experiment are believed to be valid to within 10%.

The total P* ($_tP^*$) having thus been obtained, the inorganic P* ($_iP^*$) may be determined from the supernatant after the suspension has equilibrated with the cold $H_2PO_4^-$ for ~ 10′. Determination of $_oP^*$ from the cells is preferable, however, since the "geometry" of the counting plate is more comparable to that for the total.

In either theory under consideration of the relation of phosphate to photosynthesis, an increased rate of conversion to organic acyl-phosphate compounds is expected. Were it not for limitations of permeability, one could measure the effect merely by determination of total P* uptake, since this would be increased under the influence of light. Actually, by far the slower of the two processes is that of the initial entrance of P into the cell. Considerations discussed below will show that although it is desirable to be aware of both rates, a) the rate of entry of external radio-phosphorus ($_eP^*$) into the cell, becoming internal inorganic radio-phosphorus ($_iP^*$), and b) the rate of conversion of $_iP^*$ to internal organic radio-phosphorus ($_oP^*$), the critical quantity is the rate of conversion of $_iP^*$ to $_oP^*$.

Experiments

Grana

Grana were prepared from spinach leaves, purchased locally, via a method similar to that used by Aronoff (**1**) for the chloroplasts, except for the further centrifugation in a high-speed centrifuge (20,000 g., 10′, T = 2.5° C.). Almost all of the P* was removed by washing with KH_2PO_4 ($_oP$-compounds are very soluble in the grana matrix). Thus, no conversion to $_oP$ had occurred, and the equality of P* uptake in both light and dark indicated no effect of light (table I). The theory of Emerson *et al.* (**2**) is thus not confirmed.

Leaves

Tobacco leaves, used for chloroplast preparation, were obtained from plants grown in a greenhouse in nutrient solution, during summer months. Chloroplasts were prepared by pressing leaves with a pestle in a mortar containing ice-cold M/20 $KHCO_3$, followed by differential centrifugation, and washing with distilled water.

An excised tobacco leaf was permitted to absorb P* in the dark, followed by an equal period of distilled water absorption, including three changes of

TABLE I

EFFECT OF LIGHT ON P* UPTAKE DURING PHOTOCHEMICAL REDUCTION BY GRANA*

SUBSTRATE	C/M P*	GAS EXCHANGES
xs quinone, light	3620	+86 cmm.
xs quinone, dark	3860	− 4 cmm.
No substrate, light	3660	+12 cmm.
No substrate, dark	3660	+ 6 cmm.

* For grana preparation, see text. Grana washed once and resuspended in distilled H_2O. Atmosphere, nitrogen.

water. Half the leaf was then illuminated, the stem being immersed in distilled water. From table II we note that the leaf punches show slightly more activity in the illuminated than in the dark side, but the chloroplasts, isolated from each half (2° C., as simultaneously as possible) possessed not only approximately equal activity, but they indicate no preferential conversion of ${}_{i}P^* \rightarrow {}_{o}P^*$.

If one accepts the percent of total volume occupied by the chloroplasts of a tobacco leaf as equal to a spinach leaf, i.e. 17% (MENCKE 7), then the specific activities of the chloroplasts were only ca. 1/10 that of the cytoplasm. If the leaf is half-illuminated directly while absorbing P*, a much higher P* ($\sim$2.5 times) is found on the illuminated than the dark side, although the ratio of chloroplast P*/total P* is equal for each half. This effect is presumably due to transpirational differences.

It is often stated, even among physiologists, that the use of algae is preferable to leaves, since they are more uniform, statistically, than different leaves. The comparison is not valid, since the chloroplasts within a single leaf are probably as uniform as the algae within a culture, and it is doubtful whether two algal cultures are more identical than opposite leaves. Furthermore, algae are complicated by a completeness and complexity of physiology not present within a more specialized organ such as a leaf. Thus, although a variety of experiments have been done with algae, differences in behavior of different cultures have made comparisons in experiments difficult, and the data offered are to be considered only as preliminary.

TABLE II

EFFECT OF LIGHT ON P* UPTAKE IN TOBACCO CHLOROPLASTS*

MATERIAL	LIGHT	DARK
Leaf	373.000	340,000
Total P* in chloroplasts	5,000	6,200
${}_{o}P^*$ in chloroplasts	570	620

* Leaf used ½ hour after cutting. Area = 300 cm.^{2} Stem immersed in 5 cc. distilled H_2O containing 9.0×10^6 "counts" P*. In dark 45′, after which P* solution is replaced with 20 cc. distilled H_2O, let stand 15′ and twice repeated. One half covered with Al foil. Leaf illuminated 30′ with 300 W reflector spot lamp. Illumination intensity at leaf ca. 10,000 lux. FS 311 infra-red absorbing filter between source and leaf.

TABLE III

EFFECT OF LIGHT ON P* UPTAKE BY ANAEROBIC ALGAE. 0.120 cc. ALGAE. $_{o}P^{*} = 0.6\gamma$, $C/M = 6.0 \times 10^{3}$. $t = 23'$. GAS PHASES $N_2 + 4\%$ CO_2. MEDIUM 0.1 M $KHCO_3$

	TOTAL P	$_{o}P^{*}$	% $_{o}P^{*}$
Light	101,000	38,000	38%
Dark	102,000	38,000	38%

ALGAE

The primary comparison to be made is that of light vs. dark. Furthermore, since respiration is known to involve considerable phosphorylation, while photosynthesis requires only minute amounts of oxygen, most experiments were anaerobic.

Chlorella vulgaris, grown in Emerson's solution over two 100 W white fluorescent lights (10 cm. from culture, fan circulation of air between lights) were generally 3–4-day-old cultures, which had been stored after growth at 2–3° C. until use. Because of variabilities in growth and duration of storage, the algae proved to be rather unpredictable both as to length of induction period for photosynthesis and, to a lesser extent, respiration. This variability appeared to affect both rate and extent of P* pickup so that experiments were directly comparable only if algae had been aliquoted from the same stock. Further work undoubtedly requires algae cultured and used under identical conditions. A simplified method for continuous culture has been developed and will be published shortly. A typical experiment testing the effect of light is summarized in table III. It is apparent that no effect of light is found.

Furthermore, even in the presence of oxygen, which could conceivably be specifically coupled with the phosphorylations, a short-run correlation could not be found, as is evident from table IV. Identical experiments with cultures in the presence of oxygen (or for protracted periods [60′] in its absence) indicated that 20 minutes was generally required for equilibration between $_{i}P$ and $_{o}P$ (i.e., in algae, $_{i}P/_{o}P = \frac{1}{4}$, so that when the % ratio is 20/80, complete equilibration has occurred and the continuation of the experiment beyond this time is of no further value (e.g. fig. 1).

It is, however, apparent from table III that the permeability is the limiting factor, since less than 2% of the original activity had entered the cell. To calculate the distribution between cells and medium at equilibrium,

TABLE IV

EFFECT OF LIGHT ON P* UPTAKE BY AEROBIC ALGAE. 0.100 cc. ALGAE. $_{o}P^{*} = 0.6\gamma$, $C/M = 3 \times 10^{6}$. $t = 15'$. GAS PHASES, AIR + 4% CO_2

	TOTAL P	$_{o}P^{*}$	% $_{o}P^{*}$
Light	16,300	8,200	50%
Dark	17,300	8,700	49%

assume that P enters only by exchange. Equilibrium will then occur when the specific activity of ${}_eP^* = {}_iP^* + {}_oP^*$. The average composition of algal P may be assumed to be ~ 0.1% of the wet weight, so that 120 mg. of algae contains 120 γ of P. The external radioactive solution contained 0.60 γ P, so that at equilibrium, the "count" in the external solution should be $\left(\frac{0.60}{0.60+120}\right)(6.0\times10^{-6}) = 5.0\times10^{-4}$, while the cells should contain (120/120.6) $(6.0\times10^{7}) = 6.0\times10^{7}$. It is obvious that the cells are far from equilibrium with the external solution and that the permeability is by far the slower rate.

There is a very definite effect of oxygen in the dark, as shown in table V. The data for ${}_oP^*$ appear to be somewhat high and indicate in this instance incomplete removal of ${}_iP^*$. It is to be noted that not only does the presence of oxygen accelerate the rate of conversion of ${}_iP^* \rightarrow {}_oP^*$, but also the permeability of the cell, i.e., ${}_eP \rightarrow {}_iP$. Although anaerobiosis does not always affect the rate of absorption of ions through cell membranes, there are numerous instances of this as reported by HOAGLAND (5), where it is believed to occur.

TABLE V

EFFECT OF OXYGEN ON P* UPTAKE BY ALGAE. 0.150 cc. ALGAE. ${}_eP^* = 6\gamma$, C/M = 10^6. t = 30′. GAS PHASES, $N_2 + 4\%$ CO_2; ARGON, AIR – CO_2

	TOTAL P	${}_oP$	% ${}_oP$
$N_2 + 4\%$ CO_2	23,900	11,000	46%
Argon	26,800	12,000	45%
Air – CO_2	142,000	127,000	89%

An attempt was therefore made to determine the relation between O_2 assimilation and P fixation. Duplicate flasks were used, one for the determination of respiratory pickup of P, another for fermentative, i.e., in the presence of argon. Respiratory rate was determined in a third vessel. Aliquots were made from the flask containing air + P* at 5′ intervals and ${}_oP^*$ determined. At the conclusion of the experiment, a similar analysis was made for the vessel containing argon.

One may calculate the relation between O_2 consumption + P conversion from the rate-formula given herewith. It has been shown earlier that the rate of entry of P* from the external medium into the cells is very much slower than its rate of conversion within the cell to organic P*. Therefore, neglecting ${}_eP^* \rightarrow {}_iP^*$, we may write the equation for the rate of formation of ${}_oP^*$ from ${}_iP^*$ as:

$$\frac{d_oP^*}{dt} = k\left(\frac{{}_iP^*}{{}_iP} - \frac{{}_oP^*}{{}_oP}\right) \qquad (k = \text{constant})$$

where, at t = o, ${}_iP^* = {}_oP^* = 0$; furthermore ${}_iP = {}_iP^* + {}_oP^*$ at all times. Ob-

viously, when the *specific activities* ($_1P^*/_1P$ and $_oP^*/_oP$) of the two fractions are equal, $d_oP^*/dt = 0$. The solution of the differential equation is

$$_oP^* = \frac{_oP_1P}{_oP + _1P}\left[1 - e^{-k\left(\frac{_oP + _1P}{_1P_oP}\right)t}\right]$$

which may be rewritten as

$$-\ln\ (1 - u_oP^*) = ukt. \text{ where } u = \frac{_oP + _1P}{_oP_1P}$$

From the curve (fig. 1) it is seen that half of the conversion of $_1P^* \rightarrow _oP^*$ (i.e., $_oP^* = 40\%$) occurs at 7.5 minutes. Substitution in the above, after utilization of the relation, $_1P = _1P^* + _oP^*$, results in the evaluation of $k = 14.5\%/min.$

The 290 cmm. of cells are estimated to contain 290 γ P of which 80% or 232 γ, are believed to be organic (EMERSON *et al.* **2**). This is equivalent to $232/290 = 0.80$ γ $_oP$/cmm. cells, or 0.0258 micromoles/cmm. cells. The fraction formed is $14.5\% = 0.0038$ micromoles/min./cmm. cells. The rate of O_2 assimilation is 0.015 cmm. O_2/min./cmm. cells. This is equivalent to $0.015/22.4 = 0.0067$ micromoles/min./cmm. cells. The ratio of O_2/P is thus 67/38 or approximately one atom 0/three atoms P.

Discussion

It is evident from the experimental data presented that there is no direct connection between light and the gross formation of organic phosphorus compounds. Thus the scheme of Ruben-Lipmann is not applicable in its largest interpretation. There still remain two possible interpretations by which phosphorus might still play a direct part in photosynthesis. If an organic phosphorus fraction constituting less than 10% of the total organic phosphorus were involved in the photochemical interchange with inorganic phosphorus, the present method would not have been able to detect an acceleration of this interchange by light. The second possibility is that the light accomplishes transformation of organic phosphorus from one form to another without passing through the inorganic state.

Summary

Using radioactive phosphorus, no direct connection between *gross* formation of organic phosphorus compounds and photosynthesis or photochemical reductions has been found to occur.

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CHANGES IN CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY OF DEVELOPING TUNG FRUIT WITH REFERENCE TO OIL SYNTHESIS

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Introduction

In a previous study (**26**) of changes in chemical composition of tung fruit from June 28 through November 29, 1940, it was shown that oil, sucrose, and protein accumulated in tung kernels and that starch and reducing sugar disappeared towards the end of this period. Appreciable quantities of nitrogen and carbohydrates disappeared from the hull as the fruits approached maturity and probably were in part translocated to the kernel to contribute to the substances stored there. However, the reserves lost from the hull accounted for only a small part of those stored in the kernel; hence the oil, sucrose and protein must have been largely synthesized from substances translocated to the kernels from the leaves or from storage tissues of the tree. In the investigation to be described in this paper as in the study previously reported (**26**), periodic analyses were made of developing fruits to determine the amounts of accumulated oil, and changes in dry matter, carbohydrates and nitrogenous constituents. In addition data were collected concerning: (a) the mineral, pectic acid and tannin contents of the fruit, (b) the characteristics of the oil, (c) the photosynthetic activity of the leaves on bearing shoots, (d) the catalase and respiratory activity of the fruit.

The oil present in mature tung kernels is composed largely of the glyceride of alpha-elaeostearic acid (**20**) in which the fatty acid radicals contain a conjugate system of double bonds, not found in most vegetable oils. As pointed out previously (**26**), the reducing sugar and starch are the main sources of carbon for this oil. It has been noted by other investigators (**11, 15, 17, 18, 23, 27**) that fats and oils are formed from carbohydrates but no satisfactory account is available of the exact mechanism of transformation of carbohydrates into oil (**10**). A knowledge of the biological activity and changes in composition of the different parts of the tung fruit during development is desirable because it provides fundamental information concerning the synthesis of oil in tung and because it may ultimately lead to a better understanding of the effects of orchard practices on formation of the oil.

Materials and methods

SAMPLES OF FRUIT.—In order to eliminate the wide variations in fruits due to genetic differences among seedling trees, the study was conducted through-

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out the summer and fall on fruits from a single 29-year-old tree growing under good cultural conditions on the grounds of the Florida Agricultural Experiment Station at Gainesville, Florida. Two random samples of 15 fruits each were picked at monthly intervals from May 15 to November 15. Insufficient fruits were available to permit using larger samples and a moderate sampling error was necessarily involved (**16, 24**). Fruits were picked at random from all parts of the tree between the hours of 8:00 and 10:00 in the morning. The stage of development of the fruits and their

TABLE I

STAGES OF DEVELOPMENT OF TUNG FRUITS, WITH AVERAGE WEIGHTS AND DIAMETERS ON THE SEVERAL SAMPLING DATES

SAMPLING DATE 14TH TO 16TH OF	TIME AFTER FULL BLOOM	AVERAGE DIAMETER OF FRUITS	AVERAGE DRY WEIGHT PER FRUIT	CONDITION OF FRUIT
	weeks	*centimeters*	*grams*	
May	5	2.2	1.95	Fruits succulent; integuments of the ovule soft. Embryo and endosperm of microscopic size.
June	9	4.6	6.97	Inner integument of the ovule (shell) definitely hardened. Seed filled with soft, watery nucellus. Endosperm and embryo still microscopic in size.
July	14	5.2	13.48	Structural development of fruit nearly complete. Shell sufficiently developed to be separated from nucellus. Developing endosperm and embryo visible in the center of the nucellus.
August	19	5.3	19.75	Structural development fully completed. The dry, fully developed kernel (endosperm and embryo) completely filled the shell, which was well formed, hard, and readily removed.
September	24	5.3	22.49	Fruit almost mature.
October	28	4.3	20.62	All fruits had fallen to the ground. Hulls were brown and hard.
November	32	4.1	18.77	Apparently the same as on October 15.

average weight and diameter on the various sampling dates are given in table I. The fruits were prepared for analysis by the procedure described in a previous publication (**26**).

ANALYTICAL METHODS.—The analyses for carbohydrates and oil content were made according to the procedure outlined by SELL, JOHNSTON, and LAGASSE (**26**) and the nitrogen, pectic acid and tannin were determined by the A.O.A.C. methods (**1**). The acid number, saponification number, specific gravity, and refractive index of the ether-extracted oil were determined as described by Jamieson (**12**). The iodine value of the oil was obtained by the

ROSENMUND-KUHNHENN method (**2, 25**) using a 5-minute reaction period. Catalase and photosynthetic activity of the leaves were determined by the methods of Heinicke and co-workers (**7, 8, 9**). The apparatus of Ulrich (**28**) was modified for the determination of the respiratory activity of whole fruit and kernels. Ash, calcium, potassium, magnesium, and phosphorus were determined by the methods outlined by DROSDOFF and PAINTER (**5**).

Results and discussion

The results of the chemical analyses and other determinations are summarized in tables II, III, IV and V. Each entry is the average of two determinations, on each of two independent samples.

ORGANIC CONSTITUENTS.—The principal components of the tung fruit are (1) outer hull, (2) inner hull, (3) shell, and (4) kernel. Excepting in May and June, when the fruits were too small to make the separation, these components were analyzed separately; but to simplify presentation of the data the outer hull, inner hull and shell have been combined and in this paper will be designated "hull." Since in this study results obtained with the dry matter, carbohydrate, and nitrogenous fractions are similar to those previously reported, only the main trends of these constituents will be presented here. The dry weight of the whole fruit and of its hull and kernel components attained a maximum in September and then declined appreciably (table II). This loss in dry weight, which occurred after the fruits dropped to the ground, has been observed consistently in several different studies. It may be due to respiration and in part to bacterial or mold action on the hull. It may be noted that the diameter of the fruits increased from 2.2 cm. in May to 4.6 cm. in June (table I) and that this rapid increase in size was accompanied by a decrease in percentage of dry matter from 24.23 to 17.32 (table II). Beginning June 15, the whole fruit and its hull and kernel components, gained steadily in percentage of dry matter until November.

Reducing sugar in the hull increased from 246 milligrams per fruit in July to 266 milligrams in August, and thereafter declined steadily until November. The very small quantities of reducing sugar in the kernel at the early sampling dates completely disappeared in October and November. Non-reducing sugar in the hull declined steadily from 633 milligrams per fruit in July to 235 milligrams per fruit in September, then fell to 9 and 3 milligrams, respectively, in October and November. Non-reducing sugar, identified as sucrose, was stored in the kernel, attaining a maximum of 160 milligrams per fruit in August and declining to 122 milligrams per fruit in November. The presence of sucrose in the kernels at maturity and its utilization in germination (**13**) indicate clearly that it is a reserve carbohydrate. Starch tended to accumulate in the hull during the last two months; but only a trace was found in the kernel at the September and October sampling dates, and none in November. The starch was largely in the shell, here included with the hull.

TABLE II

ORGANIC CONSTITUENTS OF WHOLE TUNG FRUITS AND THEIR COMPONENT PARTS, AT MONTHLY INTERVALS FROM MAY THROUGH NOVEMBER, 1941 (ALL PERCENTAGES, EXCEPT AS NOTED, ARE EXPRESSED ON A DRY WEIGHT BASIS)

PART OF FRUIT AND DATE OF SAMPLING*	DRY MATTER PER FRUIT		CARBOHYDRATES								PECTIC ACID PER FRUIT		TANNIN PER FRUIT		NITROGEN						OIL PER FRUIT	
			REDUCING SUGAR PER FRUIT		NON-REDUCING SUGAR PER FRUIT		STARCH PER FRUIT		POLYSACCHARIDES** PER FRUIT						TOTAL PER FRUIT		ALCOHOL-SOLUBLE PER FRUIT		ALCOHOL INSOLUBLE (BY DIFFERENCE) PER FRUIT			
	%‡	*gm.*	%	*mg.*	%	*mg.*	%	*mg.*	%	*mg.*	%	*mg.*	%	*mg.*	%	*mg.*	%	*mg.*	%	*mg.*	%	*gm.*
Hull:†																						
July	26.63	12.79	1.92	246	4.95	633	1.16	148	8.11	1037	6.33	810	6.43	822	1.06	136	0.22	28	0.84	107	0.0	0.0
August	29.52	14.88	1.79	266	2.99	445	0.99	147	7.47	1112	5.91	879	8.88	1321	0.61	91	0.07	10	0.54	80	0.0	0.0
September	28.26	14.24	1.29	184	1.65	235	0.99	141	7.89	1124	8.08	1151	8.28	1179	0.59	84	0.10	14	0.48	68	0.0	0.0
October	77.21	12.57	0.40	50	0.07	9	1.62	204	8.02	1008	2.94	370	2.38	299	0.62	78	0.04	5	0.58	73	0.0	0.0
November	85.79	11.29	0.23	26	0.03	3	1.49	168	8.13	918	3.63	410	2.77	313	0.64	72	0.04	5	0.61	69	0.0	0.0
Kernel:																						
July	8.49	0.69	2.49	17	10.10	70	0.33	2	2.36	16	0.0	0	0.0	0	2.54	18	1.86	13	0.72	5	3.88	0.027
August	49.27	4.87	0.13	6	3.29	160	0.23	11	2.24	109	0.0	0	0.0	0	3.71	181	0.31	15	3.39	165	58.29	2.839
September	78.63	8.25	0.15	12	.....	.....	Trace	Trace	2.29	189	0.0	0	0.0	0	3.33	275	0.14	12	3.19	263	64.18	5.295
October	91.50	8.05	0.0	0	1.82	147	Trace	Trace	2.02	163	0.0	0	0.0	0	3.22	259	0.10	8	3.12	251	63.83	5.138
November	95.33	7.48	0.0	0	1.63	122	0.0	0	2.35	176	0.0	0	0.0	0	3.34	250	0.08	6	3.26	244	63.19	4.727
Whole fruit:																						
May	24.23	1.95	1.79	35	2.01	39	3.79	74	9.46	184	6.20	121	17.90	349	1.91	37	0.17	3	1.74	34	.....	.....
June	17.32	6.97	3.18	222	5.15	359	2.45	171	9.77	681	10.25	714	10.15	707	1.52	106	0.26	18	1.26	88	.....	.....
July	23.99	13.48	1.95	263	5.22	703	1.11	150	7.81	1053	6.01	810	6.10	822	1.14	154	0.30	41	0.83	112	0.20	0.027
August	32.73	19.75	1.38	272	3.06	605	0.80	158	6.18	1221	4.45	879	6.69	1321	1.38	272	0.13	25	1.24	245	14.37	2.839
September	36.95	22.49	0.87	196	1.04	235	0.63	141	5.84	1313	5.11	1151	5.24	1179	1.60	359	0.12	26	1.47	331	23.54	5.295
October	82.20	20.62	0.24	50	0.76	156	0.99	204	5.68	1171	1.79	370	1.45	299	1.63	337	0.06	13	1.57	324	24.92	5.138
November	89.43	18.77	0.14	26	0.67	125	0.90	168	5.83	1094	2.18	410	1.67	313	1.72	322	0.06	11	1.67	313	25.18	4.727

* 14th to 16th of each month.
** Acid hydrolyzable residue from starch determinations.
† Includes outer hull, inner hull, and shell.
‡ Fresh weight basis.

Although on July 15 the kernel consisted largely of watery nucellus, 0.027 grams of oil were present. A period of active oil synthesis followed and by September 15, when the endosperm had filled the shell (**19**), each fruit contained on the average 5.295 grams of oil, which constituted 64.18 per cent of the dry weight of the kernels. Thereafter the weight of oil per fruit and the percentage of oil in the kernels declined slightly. The characteristics of the oil (table III) indicate that from the first it consisted almost wholly of the glycerides of elaeostearic acid. Even in the early stages of oil synthesis no appreciable quantity of free acid accumulated, and only insignificant changes occurred in the chemical and physical constants, such as the acid number, saponification number, iodine number, refractive index, and specific gravity of the oil. Thus the synthesis of oil in tung differs somewhat from that reported in macadamia (**15**), flax, rape, and hemp (**11**).

Both alcohol-soluble and insoluble nitrogen in the hull showed a general downward trend from July to November. In the kernel alcohol-soluble ni-

TABLE III

THE CHARACTERISTICS OF TUNG OIL IN RELATION TO STAGE OF FRUIT DEVELOPMENT

DATE 14TH TO 16TH OF	ACID NUMBER	SAPONIFICATION NUMBER	IODINE NUMBER	REFRACTIVE INDEX (N_D^{25})	SPECIFIC GRAVITY
August	0.93	194.4	151.8	1.5142	0.9318
September	0.00	198.3	151.3	1.5134	0.9313
October	0.00	206.2	151.3	1.5135	0.9322
November	0.00	205.8	153.4	1.5140	0.9322

trogen declined from 13 milligrams per fruit in July to 6 milligrams per fruit in November; but insoluble nitrogen accumulated, attaining a maximum of 263 milligrams per fruit in September and thereafter declining slightly. Protein, calculated from the insoluble nitrogen content, made up 19.94 per cent of the mature tung kernel as of September 15. Accordingly the three principal reserves of tung kernels are sucrose, protein, and oil. The oil, which ordinarily ranges from 60 to 70 per cent of the dry weight of the kernels, constitutes a relatively large proportion of the total carbon and total energy. The loss of reducing sugar and starch from the kernels and of reducing sugar, non-reducing sugar, and nitrogen from the hulls during the period of active oil synthesis, suggests that these substances are utilized in the formation of oil and other reserves of tung kernels. However, the substances lost from the hull probably were in part consumed in respiration or used by bacteria and molds. Since there is a small amount of starch in the kernel early in its development, it would appear that some sugar is condensed to starch at that period. Later this starch is probably converted to oil or other reserves and thereafter the reducing sugar is converted to oil, sucrose, or protein, without transformation to starch. These findings are in agreement with work already published on tung (**26**) and on pecan (**27**).

Polysaccharides (acid hydrolyzable residue from starch determinations) were abundant in the hull, reaching a maximum of 1124 milligrams per fruit in September, then declining to 918 milligrams in November. Relatively small quantities were found in the kernel, the maximum being 189 milligrams per fruit in September. On a percentage basis the polysaccharides in both hull and kernel were remarkably uniform throughout their development, the percentages in the hull ranging from 7.47 to 8.13 and those in the kernel from 2.02 to 2.36. The rate of accumulation and the distribution of the polysaccharides suggest that they are constituents of the structural materials, which are much more abundant in the hull than in the kernel. This view is supported by evidence that polysaccharides are not used in germination (**13**).

The pectic acid in tung fruit was found only in the hull. It increased from 810 milligrams per fruit in July to 1151 milligrams per fruit in September, and then declined suddenly to 370 and 410 milligrams per fruit respectively in October and November. The structural elements of the hull were complete in August but there was a marked increase in pectic acid content of the hull between August and September. This may be the result of the thickening of the cell walls with pectic substances. The loss of 781 milligrams of pectic acid per fruit between September and October may have been due to hydrolytic decomposition of the pectin into simpler compounds (**3**), or to a transformation to more complex materials, such as structural polysaccharides. At its maximum on September 15, pectic acid constituted 8.08 per cent of the dry weight of the hull.

Like the pectic acid, the tannin of tung fruit occurred only in the hull. It attained a maximum of 1321 milligrams per fruit, which constituted 8.88 per cent of the dry matter of the hull in August. The quantity decreased to 1179 milligrams per fruit in September and then dropped suddenly to 299 milligrams per fruit in October. The decrease in tannin content during the period between August 15 and September 15, when considerable oil, sucrose, and polysaccharides and proteins were being synthesized in the kernels, suggests that tannin may be hydrolyzed to simpler substances and converted to reserves. M'CLENAHAN (**21**) believes that the oil in black walnuts is derived from tannin. However, the principal drop in tannin content of the hull took place after the fruit fell to the ground and may be due simply to leaching out of the tannin after the death of the cells of the hull, or to transformation of the tannin to a less soluble form.

INOGRANIC CONSTITUENTS.—The weight of ash in the hull of each fruit increased steadily from July to October and that in the kernel from July to September (table IV). The maximum ash content of the whole fruit, 1181 milligrams, occurred in October. The ash content of the components and of the whole fruit declined slightly in November. In July, when the kernel was very watery, the ash content attained 13.46 per cent dry basis. Of the total ash in the whole fruit on November 15, 75.74 per cent was found in the hull. This preponderance of ash in the hull may be related to the

presence of a large quantity of organic acid in the hull, which would form salts with the various basic elements.

Between July and November, calcium in the hull ranged from 38 to 48 milligrams per fruit but showed no significant trend. Only five milligrams of calcium per fruit were present in the rather watery kernel on July 15, but thereafter calcium in the kernels remained constant at 18 to 19 milligrams. During the period August to November inclusive, the percentages of calcium in the hull, 0.27 to 0.40, were quite comparable to those in the kernel, 0.23 to 0.36. In July the kernel contained 0.66 per cent calcium, dry weight

TABLE IV

MINERAL CONSTITUENTS OF WHOLE TUNG FRUITS AND THEIR COMPONENT PARTS, AT MONTHLY INTERVALS FROM MAY THROUGH NOVEMBER 1941 (DRY WEIGHT)

PART OF FRUIT AND DATE OF SAMPLING*	ASH PER FRUIT		CALCIUM PER FRUIT		MAGNESIUM PER FRUIT		PHOSPHORUS PER FRUIT		POTASSIUM PER FRUIT	
	%	mg.	%	mg.	%	mg.	%	mg.	%	mg.
Hull:†										
July	4.12	527	0.34	43	0.13	17	0.15	19	1.34	171
August	5.06	753	0.28	42	0.10	15	0.10	15	1.50	223
September	5.92	843	0.27	38	0.07	10	0.13	19	2.08	296
October	7.34	923	0.38	48	0.09	11	0.07	9	1.80	226
November	6.78	765	0.40	45	0.11	12	0.08	9	2.19	247
Kernel:										
July	13.46	93	0.66	5	0.26	2	0.32	2	2.93	20
August	3.98	194	0.36	18	0.30	15	0.59	29	0.78	38
September	3.17	261	0.23	19	0.31	26	0.43	35	0.56	46
October	3.20	258	0.24	19	0.31	25	0.48	39	0.62	50
November	3.28	245	0.25	19	0.32	24	0.56	42	0.67	50
Whole fruit:										
May	4.97	97	0.63	12	0.25	5	0.26	5	1.30	25
June	5.20	362	0.64	45	0.23	16	0.17	12	1.35	94
July	4.60	620	0.36	48	0.14	19	0.16	21	1.42	191
August	4.79	947	0.30	60	0.15	30	0.22	44	1.32	261
September	4.91	1104	0.25	57	0.16	36	0.24	54	1.52	342
October	5.73	1181	0.32	67	0.17	36	0.23	48	1.34	276
November	5.38	1010	0.34	64	0.19	36	0.27	51	1.58	297

* 14th to 16th of each month.
† Includes outer hull, inner hull, and shell.

basis. The data on whole fruit indicate that calcium was relatively abundant on a percentage basis in May and June when the tissues were rather succulent.

Magnesium in the hull showed a slight downward trend from 17 milligrams per fruit in July to 10 milligrams per fruit in September and thereafter remained relatively constant. Magnesium in the kernel increased steadily from 2 milligrams in July to 26 milligrams in September, suggesting a translocation from the hull. The percentage of magnesium in the kernels, 0.26 to 0.32, was much higher than that in the hulls, 0.07 to 0.13.

Potassium was especially abundant in the hulls, increasing steadily from 171 milligrams per fruit in July to 296 milligrams per fruit in September,

after which it declined to 226 and 247 milligrams per fruit in October and November, respectively. In November the potassium content of the hulls was 2.19 per cent. In July there were 20 milligrams of potassium in the nucellus and the relatively small endosperm and embryo of the kernel. Thereafter the quantity of potassium increased steadily to 50 milligrams per fruit in October and November. The percentage was very high in July,

TABLE V

RESPIRATORY ACTIVITY OF WHOLE TUNG FRUITS* AND THEIR COMPONENT PARTS, AT MONTHLY INTERVALS FROM JULY THROUGH NOVEMBER (CATALASE ACTIVITY FROM MAY THROUGH NOVEMBER); AND THE CONCURRENT PHOTOSYNTHETIC ACTIVITY OF LEAVES ON BEARING SHOOTS

PART OF FRUIT AND SAMPLING DATE†	RESPIRATORY EXCHANGE CO_2 PER FRUIT PER HOUR AT 25° C.	RESPIRATORY EXCHANGE O_2 PER FRUIT PER HOUR AT 25° C.	RESPIRATORY QUOTIENT $\frac{CO_2}{O_2}$	CATALASE ACTIVITY O_2 PER FRUIT PER MINUTE AT 20° C.	APPARENT PHOTOSYNTHETIC ACTIVITY OF LEAVES ON BEARING SHOOTS CO_2 PER 100 SQ. CM. PER HOUR
	milliliters	*milliliters*	*ratio*	*milliliters*	*milligrams*
Hull:					
July	4.4	5.4	0.8	216	.....
August	4.4	4.4	1.0	133	.....
September	2.0	1.8	1.1	51	.....
October	3.7	4.4	0.8	4	.....
November	3.0	2.8	1.1	2	.....
Kernel:					
July	2.5	2.6	1.0	67	.....
August	3.7	3.5	1.1	72	.....
September	5.3	3.2	1.7	43	.....
October	1.7	1.4	1.2	30	.....
November	0.4	0.4	1.0	13	.....
Whole Fruit:					
May	.....	.....	.....	88	3.80
June	.....	.....	.....	150	3.13
July	6.9	8.0	0.9	283	2.77
August	8.1	7.9	1.0	205	2.65
September	7.3	5.0	1.5	94	1.44
October	5.4	5.8	0.9	34	0.91
November	3.4	3.2	1.1	15	0.70

* The fruits used for determining respiratory activity were taken from a second tree. They were equivalent in oil content and in physiological stage of development to those used for the study of composition.

† 14th to 16th of each month.

2.93 dry basis, but thereafter ranged from 0.56 to 0.78 with no significant trend. The reason for the high potassium content of the hull is not known. Potassium is believed to play a role in protein synthesis and often is concentrated at or near active meristematic regions. However, after July there is but little growth of the hull and there is no evidence of protein synthesis. Possibly it acts as a buffer for organic acids. The accumulation of potassium

in the hull parallels that of pectic acid between July and September and preliminary tests have indicated that much of the potassium in the hull is present as the salt of pectic acid. However, potassium remained at a high level in the hulls in October and November whereas the pectic acid dropped to relatively low levels at the last two sampling dates.

In contrast to the basic elements, phosphorus in the hull declined from 19 milligrams per fruit in July to 9 milligrams per fruit in October and November, whereas in the kernel it increased from 2 milligrams per fruit in July to 42 milligrams per fruit in November. Phosphorus constituted only 0.08 per cent of the hull in November, but 0.56 per cent of the kernel at the same date. The phosphorus content of seeds is commonly high. It is probably utilized in the formation of phospholipides, phosphoproteins and hexosephosphate. The pronounced increases in phosphorus, magnesium, and potassium in the kernels during the filling period, suggest that considerable amounts of these elements may be necessary for development of the kernel and its reserves.

RESPIRATORY ACTIVITY.—The exchange of respiratory gases by the kernel and the whole fruit was measured at monthly intervals from July through November. The respiration of the hull was obtained by difference between that of the kernel and that of the fruit after calculating the observed values of gas exchanged on a per fruit basis. The data in table V show the average number of milliliters of carbon dioxide and of oxygen exchanged per fruit, per hour, on the various dates. The respiratory activity of the hull declined toward the end of the season, yet in October and November the rates of exchange of carbon dioxide and of oxygen were more than half those during peak respiratory activity, in July and August. Since in October and November the hulls were "brown and hard," it is quite possible that the respiration observed was at least in part that of micro-organisms. The data on respiration support the previous conclusion that after the fruit drops from the tree, the hull loses weight through respiration of its tissues and in part through the action of fungi and bacteria. At all times the respiratory quotient of the hull was close to unity.

The rate of carbon dioxide evolution of the kernels was at a maximum in September; the rate of oxygen assimilation was high, but lower than in August. This resulted in a high respiratory quotient, which might ensue from the release of oxygen incident to the conversion of glucose into the elaeostearic acid glyceride, the principal component of tung oil. However, the maximum accumulation of oil in the kernel occurred between July 15 and August 15, respiratory activity on the July and August sampling dates was considerably lower than in September, and the respiratory quotient was at or near unity. A high respiratory quotient when the synthesis of oil was at the maximum and reducing sugar content at its minimum, was observed by GERBER (**6**) in the seeds of castor beans, almonds, and rape. A consistently high respiratory quotient throughout the period of oil formation

would be further evidence that the oil in tung kernels is derived from carbohydrates.

CATALASE ACTIVITY.—The catalase activity (table V) of the hull was at its maximum, 216 milliliters of oxygen per fruit per minute, in July and declined steadily to four and two milliliters of oxygen per fruit per minute respectively in October and November. This appears in general to parallel the metabolic activity of the hull and would further support the hypothesis that the apparent respiration of the hull observed in October and November is due primarily to micro-organisms. Catalase activity in the kernel attained a maximum of 72 milliliters of oxygen per fruit per minute in August and then declined steadily to 13 milliliters of oxygen per fruit per minute in November. This trend would seem to parallel metabolic activity in the kernel very closely, the most active accumulation of reserves occurring in August, whereas by October and November the embryo and endosperm had entered a resting stage. The trend in catalase activity was somewhat similar to that of respiratory activity, excepting that catalase activity was relatively greater in July and August.

PHOTOSYNTHETIC ACTIVITY OF LEAVES ON BEARING SHOOTS.—Probably most of the carbohydrates translocated to the kernels (**22**) and there utilized for the formation of oil (**26, 27**) come directly from current supplies elaborated in the leaves. The data in table V show that the highest rates of photosynthetic activity of leaves occurred in May and June during the early stages of fruit development. At that period active shoot growth (**15**) and rapid increase in the size of the fruit are taking place simultaneously. It is reasonable to assume that in July and August a large amount of the products of photosynthesis is required to form oil and other reserves in the kernels. Also some increase in thickness of the trunks and branches is taking place at that time, and in addition considerable quantities of reserves, particularly starch and sucrose, are being stored in the tissues of the tree itself. The rate of photosynthetic activity continued high during July and August but decreased markedly at the approximate time that the fruit had attained maximum oil content.

Summary and conclusions

1. The whole fruit and its hull and kernel components (includes inner hull, outer hull, and shell) gained consistently in dry weight until September, but declined appreciably after the fruit dropped to the ground. The loss is probably due to respiration and in part to action of bacteria and fungi on the hull.

2. Reducing sugar in the hull increased until August and then declined. Only very small quantities were present in the kernel and these completely disappeared in October and November.

3. Non-reducing sugar increased steadily in the hull until September but almost disappeared in October and November. It accumulated in the kernel, attaining a maximum of 160 milligrams per fruit in August, there-

after declining slightly. The evidence indicates that it is a reserve carbohydrate of the kernel.

4. Starch accumulated toward the end of the season in the hull but disappeared completely from the kernel by November 15.

5. On July 15 the kernel contained 0.027 gram of oil per fruit. A period of active oil synthesis ensued, and on September 15 the kernels contained 5.295 grams of oil which constituted 64.18 per cent of their dry weight. Even in the early stages no appreciable quantity of free acid accumulated and the characteristics of the oil indicate that from the first it consisted almost wholly of the glyceride of elaeostearic acid.

6. Both alcohol-soluble and insoluble nitrogen in the hull declined steadily from July to November. In the kernel soluble nitrogen declined but insoluble nitrogen accumulated and, calculated as protein, it constituted 19.94 per cent of the mature tung kernel as of September 15.

7. Reducing sugar and starch declined in the kernel, and reducing sugar, non-reducing sugar, and nitrogen declined in the hull during the period of the filling of the nuts, which suggests that these substances are utilized in the formation of oil and other reserves of the tung kernel. Sucrose, protein, and oil are the three principal reserves of tung kernels.

8. The rate of accumulation of the polysaccharides and their distribution between hull and kernel suggests that they are constituents of the structural elements.

9. Pectic acid occurred only in the hull. Although the hull was structurally complete in August, a marked accumulation of pectic substances occurred between August and September, which suggests that cell walls were being thickened with pectic substances. A sudden loss of pectin between September and October suggests transformation into other compounds.

10. Tannin occurred only in the hull. It attained a maximum in August when it constituted 8.88 per cent of the dry matter of the hull. A considerable loss occurred between August and September, when reserves were being stored in the kernels, which suggests that some of the tannin may be hydrolyzed to simpler substances and converted to reserves. However, the principal loss in tannin content took place after the fruit fell to the ground.

11. About three-fourths of the total ash of the whole fruit was found in the hull. Calcium remained relatively constant in both hull and kernel throughout the season. Magnesium showed a downward trend in the hull and accumulated steadily in the kernel until September. Potassium was especially abundant in the hull. The preponderance of basic elements in the hull may be related to the presence of large quantities of organic acids, which would form salts with the various basic elements.

12. Phosphorus in the hull declined to a very low level and there was a concomitant increase in phosphorus of the kernel, which suggests a translocation from hull to kernel. In the kernel phosphorus may be utilized to form phospholipides, phosphoprotein, and hexosephosphate. The presence

of considerable quantities of phosphorus, magnesium, and potassium in the kernels during the filling period suggests that these elements are important in the development of the kernel and its reserves.

13. The evolution of carbon dioxide by the hull declined toward the end of the season, yet in November it was more than half the maximum rate for the season. Some of the carbon dioxide may have been evolved by microorganisms. Respiration accounts for loss in weight of the fruit after it fell to the ground. Respiratory activity of the kernel attained a maximum in September, when a respiratory quotient of 1.7 was observed. The low oxygen requirement may be accounted for on the basis of oxygen release incident to the conversion of glucose into the eleostearic acid glycerides; but this high respiratory quotient was not observed in August when oil synthesis was at its peak.

14. Catalase activity in both hull and kernel tended to parallel the metabolic activity of the respective components.

15. The highest rate of photosynthesis was observed in May and June during the stage of rapid fruit development and active shoot growth. Photosynthesis remained at a high level during July and August when reserves are being stored in the kernel, increase in thickness of the trunks and branches was taking place, and reserves were being stored in the tissue of the tree itself. Subsequent to the dropping of the fruit, the rate of photosynthesis decreased to a relatively low level.

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INFLUENCE OF LIGHT INTENSITY UPON THE CONCENTRATION OF THIAMIN AND RIBOFLAVIN IN PLANTS[1]

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It is a well known fact that the concentration of ascorbic acid and carotene is greater in plants grown in high light intensity than in low light intensity (**5, 6**). The knowledge of the effect of light on thiamin and riboflavin is very imperfect and almost negligible (**7**). In a previous paper (**4**) the writer noted that as the season changed from winter to spring and the light intensity increased there was an increase in the concentration of these two vitamins in the tomato plants. These observations were, however, incidental, and the present investigation was set up to study the influence of the intensity of natural light more carefully.

Methods and Material

The thiamin and riboflavin have been determined by the fluorescence method as set up by CONNOR and STRAUB (**1, 2**). The procedure as applied to green plants has already been described by the author (**4**). As source material for the vitamins tomato, corn, pea, bean, New Zealand spinach (*Tetragonia expansa*) and potato plants have been used. The plants of tomato, potato and New Zealand spinach were several months old, whereas corn, bean and pea plants were only seedlings. In some experiments the influence of chlorophyll was studied and in these experiments albino corn was used. The plants were all grown in the greenhouse or in a darkroom and the light intensity was varied by enclosing the plants in large cages covered with unbleached muslin cloth of various thicknesses to give the desired difference in light intensity. It has been impossible to use light of constant intensity, but as plants from different light levels were used on the same day direct comparisons can be made.

The experiments were performed over a period of months so that different light intensities were encountered. However, some of the determinations were made during the summer when the light intensity was high and more nearly constant and for this reason not only the determinations within an experiment but also data from different experiments can be compared when the plants were of the same age.

Results

In the first experiments two extremes of light intensity were investigated, i.e., darkness and full daylight, and tomato plants were employed

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as plant material. A number of similar plants were selected and some were placed in the darkroom and others in the greenhouse. Usually a second lot of the same plants was placed in the darkroom several days later than the first group so as to have plants that had been in the darkroom different lengths of time. The darkroom was next to the greenhouse and the air for its ventilation was taken from this greenhouse. The temperature was therefore essentially the same in the two rooms. These experiments (table I) show that the vitamin content decreased rapidly when the plants were in the darkroom. In mature leaves the decrease was less rapid than in immature leaves. When the plants that had been

TABLE I

INFLUENCE OF DARKNESS ON THIAMIN AND RIBOFLAVIN CONCENTRATION IN TOMATO PLANTS. FIGURES WITHIN THE BRACKETS DENOTE THE NUMBER OF DAYS THE PLANTS HAVE BEEN IN DARKNESS. VITAMIN CONTENT IS DENOTED IN MICROGRAMS PER GRAM OF FRESH PLANT MATERIAL

Date	Exp. No.	Data on the plants	Thiamin Content			Riboflavin Content		
			Plants in dark		In light	Plants in dark		In light
2/27	45	31–42 in. tall, 3 leaves near top	1.00 (5)	1.11 (3)	1.23	0.86	0.78	0.98
3/3	46	Apices from plants in 45	1.15 (9)	1.50 (7)	1.54	2.09	2.08	2.40
3/6	47	20–22 in. tall, apices used	1.03 (6)	1.30 (3)	1.46	1.02	1.26	1.39
3/13	49	Same plants as in 47, two upper leaves	0.58 (13)	0.54 (10)	0.73	0.88	1.04	1.33
7/5	77	58–62 in. tall, apices	3.01 (9)	.....	3.52	0.62	.....	1.26
		Mature leaves	0.51 (9)	.....	0.58	0.69	.....	0.96
7/25	82	2 top leaves from plants used in 77 after 20 days in light	1.34	.....	1.08	1.30	.....	1.15

in the dark for several days were put back in light and left there for some time there was an increase in the vitamin in these plants. This is important as it demonstrates that the plants in the dark had not been injured and as soon as they were again exposed to light there was an increase in vitamin synthesis.

An examination of the table might lead one to the conclusion that the determinations were not very accurate as sometimes the concentrations were greater and sometimes smaller, in different experiments even when the plants employed were the same. This difference is due to the origin of the leaves and the age of the plants. In a previous publication (**4**) it was mentioned that there is a concentration gradient in the tomato plant; the mature leaves have the lowest vitamin content and the apex the highest.

In the same investigation it was learned that younger plants have less vitamin region for region than older plants. The same plants were used in experiments 77 and 82, but in 77 old mature leaves were used whereas in 82 leaves just below the apex, which were not yet mature were used. In 45 and 49 leaves from essentially the same part of the plant were used but in 49 the leaves came from much younger plants and it should also be noted that in 49 the apex was removed first, whereas in experiment 45 the apex was still in place when the leaves were removed. For these reasons there can be no comparison as to quantity of vitamin between the different experiments cited in this table. However, all experiments show that plants deprived of light lose thiamin and riboflavin.

Some of the tomato plants from which the leaves were taken were not very large and it was thought that a few days in the dark might deplete the stored food so it was decided to use seedlings which were still dependent

TABLE II

INFLUENCE OF DARKNESS ON THE CONCENTRATION OF VITAMIN IN BEAN, PEA AND POTATO PLANTS. VITAMIN CONTENT IS DENOTED IN MICROGRAMS PER GRAM FRESH PLANT MATERIAL

DATE	EXP. NO.	AGE AND KIND OF PLANT	THIAMIN		RIBOFLAVIN	
			DARK ROOM	GREEN-HOUSE	DARK ROOM	GREEN-HOUSE
3/24	51	Beans 18 days old	0.73	0.81	0.80	1.28
3/27	52	Same plants as in 51	0.69	1.77	1.28	1.25
4/24	61	Beans 12 days old	0.73	0.96	1.23	1.76
5/8	65	Beans 8 days old	0.81	1.30	0.66	1.44
5/12	66	Same plants as in 65	0.96	1.11	1.41	1.39
4/28	62	Peas 16 days old	0.73	1.00	1.18	1.73
	62	Potato vines 28 days	0.38	0.67	0.67	1.02
6/5	73	Potato vines 66 days	0.34	1.04	1.11	1.44

upon the food stored in the storage organs. Beans, peas and potatoes were chosen because they grow quickly and the plants could be used while there was still an abundance of food left, so there could be no starvation effect. The data from these experiments are recorded in table II. Again it is obvious that there is less thiamin in plants grown in the dark than in plants grown in light. The potato vines are more striking than the others, perhaps because they grew over a longer period and the difference became, as a consequence, greater. In the first experiment the thiamin concentration was low in both lots of plants, but in the second experiment the plants in the light had a very high concentration but the plants in the dark remained low as before. The riboflavin situation is a little different. In two experiments with the beans the riboflavin was actually a few hundred micrograms higher in the plants grown in the dark than in those grown in the light. In both experiments there was a large increase in the plants from the darkroom over what it had been before. It is inconceivable that this should be an error in the determination as the riboflavin analysis is easy

to make and high accuracy has always been obtained in recovery experiments.

In a study of growth hormones (3) the writer found that when albino and green corn plants were grown in light the albino plants had a much higher growth hormone content than the green corn. It was concluded that the chlorophyll might act as a photosensitizer which acted to destroy the hormone. With this in mind a variety of corn (Emerson 43–85–15), which yields albino, lutea and green plants, was grown both in the dark and light. It was not possible to distinguish the lutea plants from the green plants when grown in the dark as both were yellow, and therefore an ordinary variety of field corn was used for the chlorophll producing plants. The results are given in table III. The albino plants had a higher concentration of thiamin in the dark than in light while as usual the green plants produced more in the light. The riboflavin was again

TABLE III

INFLUENCE OF DARKNESS ON THE CONCENTRATION OF THIAMIN AND RIBOFLAVIN IN CORN PLANTS. A SELECTION OF CORN (43–85–15) WHICH YIELDS ALBINO, LUTEA AND GREEN PLANTS WAS USED AS WELL AS ORDINARY FIELD CORN. VITAMIN CONTENT IS DENOTED IN MICROGRAMS PER GRAM FRESH PLANT MATERIAL

DATE	EXP. NO.	AGE AND KIND OF CORN	THIAMIN		RIBOFLAVIN	
			DARK ROOM	GREEN-HOUSE	DARK ROOM	GREEN-HOUSE
3/24	51	Albino 14 days old	1.15	0.81	0.98	0.93
4/10	56	Albino 15 days old	0.88	0.54	.....	.....
	56	Field corn 15 days old	0.69	0.88	.....	.....
4/14	58	Albino 10 days old	1.04	1.01	1.07	1.12
4/24	61	Field corn 12 days old	0.84	0.92	1.22	1.33
5/8	65	Field corn 8 days old	1.00	1.38	1.12	1.49
7/1	75	Albino 12 days old	1.42	0.92	0.83	0.61

somewhat variable. A ready explanation of these experiments is that light destroys thiamin or it is used up more rapidly in the light, but that as a result of photosynthesis more is synthesized and green plants are richer in thiamin in the light and albino plants which do not photosynthesize are richer in the dark. This brings to mind the relation between chlorophyll and light, where it is known that chlorophyll is destroyed by light, but also synthesized by the plant in light.

Since early experiments on the influence of light showed that more thiamin was present in green plants in the light than in the dark, it became desirable to keep the plants in the light but to vary the intensity to which they were exposed. These experiments were then run concomitantly with the dark-light experiments.

The experiments were set up in a small greenhouse compartment having a westerly exposure. The different intensities were obtained by placing the plants in cages made of a wooden frame covered with unbleached

TABLE IV

INFLUENCE OF THE INTENSITY OF LIGHT ON VITAMIN CONTENT IN THE TOMATO PLANT. VITAMIN CONTENT IS DENOTED IN MICROGRAMS PER GRAM FRESH PLANT MATERIAL

DATE	EXP. NO.	AGE AND SIZE OF PLANTS AND PART USED	THIAMIN			RIBOFLAVIN		
			GREATER SHADE	INTERMEDIATE	FULL LIGHT	GREATER SHADE	INTERMEDIATE	FULL LIGHT
4/3	54	16 weeks 15–16 in. tall, 1 leaf from each plant	0.81	1.27	1.54	0.83	1.02	1.23
4/7	55	Same plants as in 54, leaf just below 54	0.73	1.07	1.27	0.99	1.10	1.07
4/11	57	Same plants as in 54 tip of plant used	1.96	2.42	2.65	1.34	1.26	1.32
4/21	60	12 weeks, sturdy plants tip of plants used	1.80	2.11	2.02	1.49	1.63	1.81
5/1	63	1 leaf from each plant just below cut for 60	1.54	1.46	1.52	1.06	1.19	1.50
5/5	64	Leaf just below that for 63	1.04	1.19	1.23	1.15	1.30	1.47

muslin to obtain the proper reduction of the light. The cages were about $3 \times 3 \times 3$ feet. The cages were placed on a north-south bench with the greater shade at the north end. The light intensity varied a great deal from day to day. As measured at different times of the day and seasons by a General Electric light meter the intensity in the greater shade was found to be on an average only 18 per cent of full light and 32 per cent in the cage with the intermediate light. The range of light in the unshaded condition varied from 250 foot candles on a rainy spring day to between 2000 and 4000 foot candles on a bright summer day.

That the quantity of light influences amount of thiamin in a number of plants is evident from tables IV and V. Again the albino plants had the least thiamin in the highest light intensity, while in the green plant the

TABLE V

INFLUENCE OF THE INTENSITY OF LIGHT ON VITAMINS IN BEAN AND CORN PLANTS. VITAMIN CONTENT IS DENOTED IN MICROGRAMS PER GRAM FRESH PLANT MATERIAL

DATE	EXP. NO.	AGE AND KIND OF PLANT	THIAMIN			RIBOFLAVIN		
			GREATER SHADE	INTERMEDIATE	FULL LIGHT	GREATER SHADE	INTERMEDIATE	FULL LIGHT
5/15	67	Beans 13 days old	0.92	1.04	1.11	1.31	1.42	1.44
5/19	68	Field corn 17 days old	0.69	0.75	0.73	0.89	1.11	1.11
5/23	70	Same plants as in 67	0.73	0.85	0.90	0.96	1.18	1.36
5/26	71	Same corn as in 68	0.61	0.54	0.73	0.83	0.88	0.94
6/11	74	Same beans as in 67 and 70	0.88	0.62	1.11	1.25	1.44	1.73
7/3	76	10 day old lutea corn plants	1.65	1.53	1.38	0.97	0.86	0.89
7/8	78	15 day old albino corn	1.38	1.00	1.30	1.12	1.11	1.20
10/27	99	New Zealand Spinach	0.19	0.31	0.35	0.58	0.69	0.69
10/31	100	New Zealand Spinach	0.37	0.38	0.46	0.64	0.80	0.82

reverse was true. In general, though again with some exceptions, the riboflavin was highest in the greatest amount of light.

The cause of the higher vitamin content in green plants grown in the light does not seem to be entirely clear. Even in plants with an abundant food supply the light increases thiamin and riboflavin of a plant. One might therefore speculate as to the influence or some labile substance produced as a result of photosynthesis. This supposition is strengthened by the fact that in old leaves, where one might expect photosynthesis to be low, the difference between plants in the dark and light is much less than in younger leaves. That light also has a destructive effect is amply demonstrated in the experiments with the albino corn, where the plants grown in the dark or under low light intensity have the highest vitamin content. The two opposite effects of light can be explained if one considers that the destructive action is direct and that the beneficial action is indirect through photosynthesis.

Summary

1. The effect of intensity of light on the concentration of thiamin and riboflavin has been studied in tomato, potato, bean, pea, corn and New Zealand Spinach plants.

2. It has been found that the concentration of thiamin in these species is greater in plants exposed to high light intensity than in plants exposed to darkness or low light intensity. In albino corn the opposite is true.

3. In general the above statement also holds true for riboflavin though the effect is less pronounced.

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BLOSSOMING AND PIGMENT CONTENT

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(WITH THREE FIGURES)

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The relations of several different physiological conditions to blossoming have been reported during a general study of the causes of flowering. These are principally, the irregular diurnal cycle of CO_2 exchange of plants which are blossoming (**5**), the reduction in cambial activity associated with blossom induction (**8**), a sudden change in growth rate when a plant is placed in an environment which brings about flowering (**2**), and the reduced root development which occurs when plants enter the reproductive state (**7**). These conditions were found consistently in flowering plants regardless of whether they were induced in a long or short photoperiod, or were neutral to day length. The present report is of a study made to determine the pigment content of non-flowering and of flowering seed-plants to determine if the pigment content is alike in all non-flowering plants and different from that of flowering plants. An investigation was also undertaken to determine if differences in pigment content are associated with the reproductive state when different branches of the same plant are caused to be flowering and non-flowering.

Methods

The chromatographic method (**9**) of measuring the pigments was used to ascertain the pigment content of mature foliage of two principal series of plants. The proper precautions needed in preparation of columns, namely, securing extracts for development and procedure during development to give uniform and consistent results, were rigidly employed. These precautions gave data which were uniformly repeatable. One-half gram samples of fresh leaf tissue were extracted with acetone followed by development with petroleum ether (B.P. 30–75° C). Columns 12 mm. in diameter were used. The chlorophyll, xanthophyll and violaxanthin (purplish) bands formed at different levels in the calcium carbonate in the upper part of the column and the carotene bands developed in the lower part of the column which consisted of a one to one mixture of magnesium oxide (Mallinchrodt U.S.P. Light) and "Hyflo Supercel" (Johns-Manville Co.). The amount of pigments was recorded after measuring the band widths with a vernier caliper.

This method gave a narrower range of values for carotene than the colorometric technique. Fifty-eight samples were eluted and the carotene content determined colorometrically by Dr. Robert MacVicar of the Department of Biochemistry. In one typical series of samples the band widths ranged from 7.0 to 12.8 mm. and the gamma of beta carotene equivalent

varied from 23.0 to 73.7. The narrower range of values for the chromatographic method arises from the relatively great width of bands when the extract has a low concentration of pigment.

Materials and results

The initial series of plants consisting of 18 species was studied in 1943–44. These plants were grown in long and short day conditions at four temperatures: continuously warm (75° minimum); warm-cool (75° for 13 hours at night and 55° for 11 hours in the day, lag not counted); cool-warm (55° at night and 75° in the day); and continuously cool (55° minimum). The list of plants included four long day types (*Chrysanthemum Leucanthemum,*

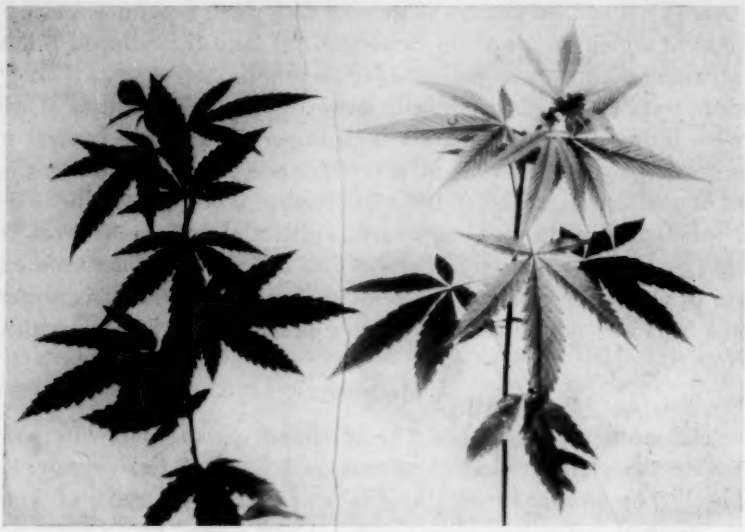

FIG. 1. Hemp (*Cannabis sativa*). The etiolating effect (right) of growing warm-loving plants, as hemp, with warm nights and cool days does not interfere with blossom induction.

Helianthus annuus, Mathiola incana var. Christmas Pink, and *Spinacia oleracea*); nine short-day types (*Amaranthus retroflexus, Cannabis sativa, Cosmos sulphureus* var. Klondyke, *Datura Stramonium, Euphorbia pulcherrima, Glycine Max* var. Biloxi, *Nicotiana tabacum* var. Maryland Mammoth, *Panicum miliaceum* var. Japanese, and *Xanthium echinatum*); and five day-neutral types (*Thlaspi arvense, Cucurbita pepo* var. Zucchini, *Lycopersicon esculentum, Nicandra physalodes,* and *Solanum tuberosum*).

A striking feature of this series of plants was the response of plants which grow best at a warm temperature. When these were given warm nights but cool days, the new growth was quite etiolated (Fig. 1). This did not, however, interfere with blossom induction. Flowering was delayed only slightly and not prevented by the marked reduction in green pigment. This response is in agreement with reports from photoperiod studies that

photoperiodism is quite independent of photosynthesis (3). Figure 2 shows data typical of a plant which blossoms in short photoperiods (Cockle-bur, *Xanthium echinatum*), another plant which blossoms in long photoperiods (Ox-eye Daisy, *Chrysanthemum Leucanthemum*), and a third plant, the day-neutral *Nicandra physalodes* which blossoms in both photoperiods when grown at different temperatures. Not only is there a lack of relation between the green pigment and flowering (Fig. 1), but also, none of the pigments shows a consistent association to the reproductive state.

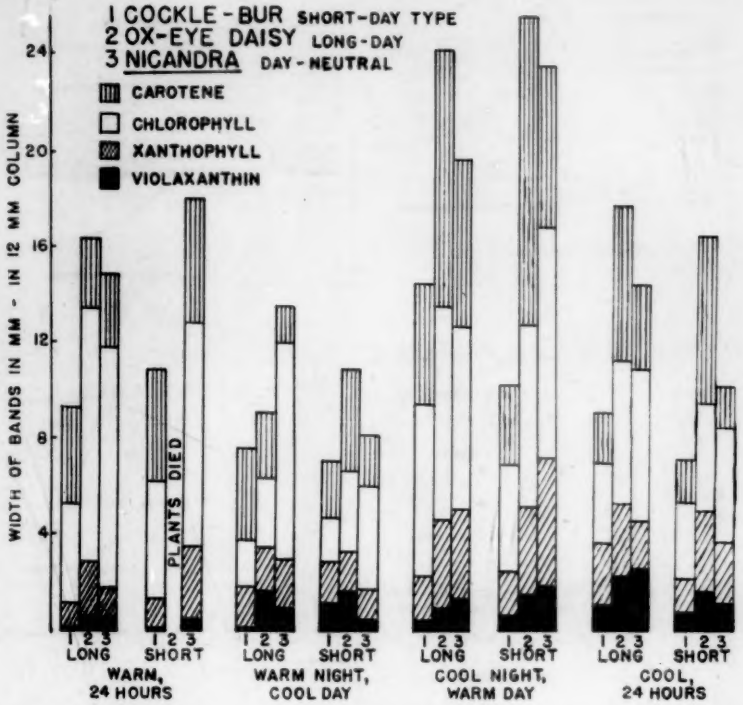

FIG. 2. Pigment content of typical short-day, long-day and day-neutral plants at different photo-period and temperature combinations. At the time of sampling, the cocklebur was in blossom in the short-day environment, the Ox-eye Daisy in the long days and all Nicandra plants were blossoming.

Another feature of the data is that the different species have characteristically different amounts of the various pigments as, for example, relatively larger amounts of chlorophyll and little carotene occurred in Nicandra and Helianthus, a small amount of both in Xanthium and Spinacia, and a large amount of both in Datura and Cosmos. Also Nicandra and Glycine contained relatively large quantities of xanthophyll while Cannabis and Glycine were high in violaxanthin. Nicandra, Datura, and Euphorbia were low in violaxanthin.

A second series of measurements were made during 1945 and 1946. These were planned to determine if the pigment content during the daily

dark period is related to blossom induction. The question arose from the obvious relation of night temperature to flowering (**4**) and from the evident lack of relation between pigment and reproduction when samples were collected during the day time (Fig. 2). Samples were collected at two-hour intervals for 24 hour periods on 29 different days. The following 15 species were sampled for daily cycle of pigment content (four long-day, eight short-day and three day-neutral types): *Cannabis sativa, Chrysanthemum frutescens, Cosmos sulphureus* var. Klondyke, *Datura Stramonium, Euphorbia*

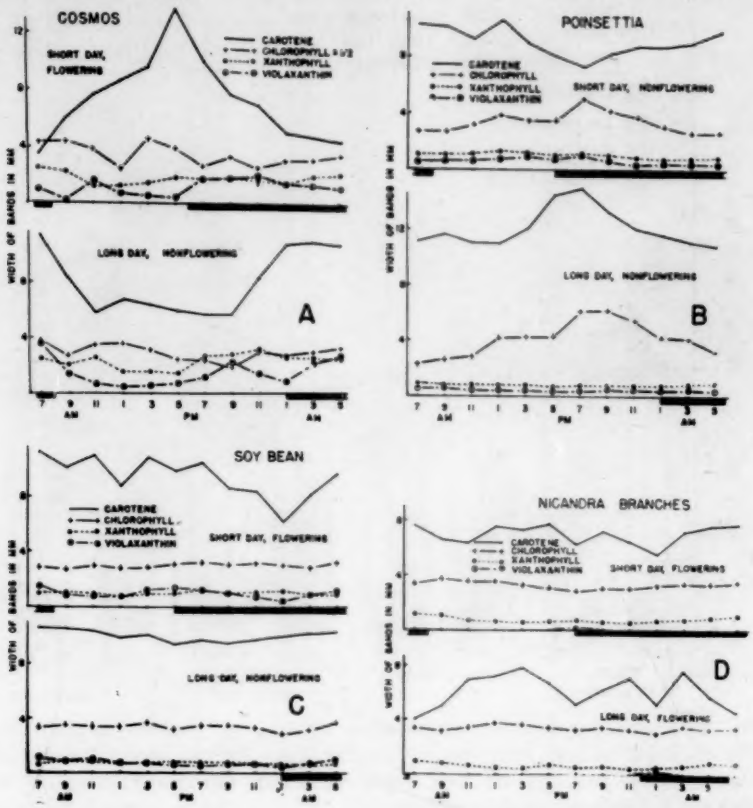

FIG. 3. Graphs showing the pigment content of different plants or branches of a single plant during 24-hour periods.

pulcherrima, Glycine Max var. Biloxi, *Helianthus annuus, Helianthus tuberosus, Nicandra physalodes, Nicotiana rustica, Penstemon Hort.* sp. var. Garnet, *Perilla nankinensis, Trifolium repens* var. Ladino, *Vicia villosa, Xanthium echinatum.* Typical sets of data are shown in figure 3. The content of chlorophyll, xanthophyll and violaxanthin show no association with the reproductive state either in the graphs of figure 3 or in the data for the other 12 species sampled. A prominent feature of the data is the pronounced diurnal cycle of carotene content in many species. The data on

TABLE I

WIDTH OF BANDS OF CAROTENE IN A 12 MM. COLUMN, 1945–6. ONE-HALF GRAM SAMPLES OF FRESH, MATURE LEAVES FROM FLOWERING AND FROM NON-FLOWERING PLANTS OR BRANCHES OF A SINGLE PLANT.

No.		Photo-period	Repro-ductive state	A.M.			P.M.						A.M.		
				7	9	11	1	3	5	7	9	11	1	3	5
	Short-day types														
1.	Poinsettia—Euphorbia pulcherrima	Short	Flow.	4.5	5.8	5.2	4.6	6.0	7.2	5.2	4.9	5.0	5.8	6.2	4.9
2.	"	Long	Non-F.	11.1	10.9	.....	10.2	6.8	4.8	5.4	6.1	7.6	7.2	9.9	10.2
3.	Cosmos sulphureus var. Klondyke*	Short	Flow.	3.5	6.0	7.6	8.7	9.6	13.7	11.1	7.7	7.0	5.1	4.8	4.5
4.	"	Long	Non-F.	11.4	8.3	5.7	6.7	6.3	5.9	5.7	5.7	8.3	10.7	10.8	10.6
5.	Hemp—Cannabis sativa-female	Short	Flow.	10.1	8.7	8.0	9.8	11.2	10.6	9.6	9.0	8.6	8.1	9.4	10.7
6.	"	Long	Non-F.	12.0	13.9	11.7	9.6	9.7	8.9	9.8	13.0	12.7	11.2	13.2	11.9
7.	Hemp—Cannabis sativa-male	Short	Flow.	8.2	6.9	4.6	9.1	9.8	10.3	11.0	9.7	10.8	7.7	10.2	8.7
8.	"	Long	Non-F.	12.8	12.7	7.0	7.8	11.6	10.2	11.3	10.5	10.4	11.8	14.0	12.9
9.	Jimson weed—Datura stramonium	Short	Flow.	7.8	7.3	9.2	10.5	6.3	5.8	7.0	4.8	4.7	5.6	5.8	6.7
10.	"	Long	Non-F.	9.9	12.8	13.6	15.0	15.2	10.4	9.2	7.6	7.8	8.0	10.4	9.8
11.	Sunflower—Helianthus annuus	Short	Flow.	5.9	6.8	5.7	5.7	6.3	5.8	5.3	5.0	6.4	7.3	7.0	6.6
12.	"	Long	Non-F.	6.9	6.8	7.0	7.7	?	8.6	7.5	6.7	5.2	5.7	6.6	6.7
13.	Perilla nankensis	Short	Flow.	6.8	7.8	6.9	5.7	5.5	5.7	7.7	7.0	8.0	7.7	6.8	5.9
14.	"	Long	Non-F.	5.4	6.7	6.9	7.2	7.0	7.9	8.3	7.7	7.8	6.8	6.0	6.0
15.	Biloxi soybean—Soja Max**	Short	Flow.	11.3	10.1	11.0	8.8	10.9	9.9	10.5	8.6	8.5	6.2	8.2	9.8
16.	"	Long	Non-F.	10.6	10.6	10.4	9.9	10.1	9.4	9.7	9.5	9.8	10.0	10.2	10.4
17.	Cocklebur—Xanthium echinatum	Short	Flow.	6.4	7.8	6.8	6.1	7.6	7.0	6.6	5.0	6.8	6.1	7.7	6.5
18.	"	Long	Non-F.	9.5	9.0	7.6	8.0	8.4	7.5	7.1	6.4	6.5	7.2	7.9	8.7
	Long-day types														
19.	Penstemon, Hort. Var. Garnet	Short	Non-F.	4.2	5.8	6.4	6.2	6.5	7.0	8.1	7.3	5.8	5.6	5.2	5.1
20.	"	Long	Flow.	6.8	7.2	5.1	9.1	7.2	7.0	6.4	6.2	7.3	8.0	7.6	7.0
21.	Ladino clover—Trifolium repens	Short	Non-F.	5.5	7.0	10.1	8.7	7.8	6.9	7.3	7.0	7.3	8.8	7.3	7.3

TABLE I.—(*Continued*)

No.	Photo-period	Repro-ductive state	A.M.			P.M.						A.M.		
			7	9	11	1	3	5	7	9	11	1	3	5
22. Ladino clover—Trifolium repens	Long	Flow.	9.0	10.9	8.4	11.4	11.8	10.1	9.0	8.6	7.7	7.6	8.2	8.7
23. Winter vetch—Vicia villosa	Short	Non-F.	7.3	8.5	7.9	7.5	7.6	9.6	9.1	7.5	8.4	9.8	9.4	8.7
24. "	Long	Flow.	8.2	12.3	8.1	9.6	12.2	9.7	10.9	10.2	9.9	10.7	8.6	8.1
25. Marguerite—Chrysanthemum frutescens	Short	Non-F.	7.9	6.4	6.4	6.7	6.1	5.7	5.8	5.8	5.2	5.8	7.1	8.2
26. "	Long	Flow.	9.5	8.0	6.2	6.8	8.7	6.3	5.9	6.0	5.5	7.6	8.8	9.3
Day-neutral types														
27. Nicandra physalodes	Short	Flow.	11.0	8.8	7.7	7.6	6.6	6.0	6.4	7.0	9.0	10.4	9.7	10.5
28. "	Long	Flow.	9.1	8.5	6.6	6.6	8.2	7.0	7.5	8.7	8.7	?	8.0	10.2
29. Tobacco—Nicotiana rustica	Short	Flow.	9.1	5.9	4.3	5.1	6.2	5.5	7.2	8.3	6.7	6.4	6.9	7.4
30. "	Long	Flow.	7.7	4.7	6.3	5.0	6.3	7.5	4.1	4.2	5.0	5.6	6.0	6.2
31. Artichoke—Helianthus tuberosus	Short	Non-F.	8.1	6.4	6.9	5.1	5.8	4.7	6.0	6.0	6.0	4.6	6.0	6.2
32. "	Long	Non-F.	8.6	7.0	6.1	5.2	?	6.3	5.8	5.9	5.0	5.0	6.4	7.6
Special treatments														
33. Jimson weed—Datura stramonium	Short	Flow.	5.3	5.9	5.0	5.5	7.0	5.6	4.1	4.8	5.4	5.9	4.8	5.6
34. " Fruiting from age	Long	Flow.	6.4	8.0	6.4	6.2	5.9	7.3	6.9	6.3	4.8	4.4	4.6	6.1
35. Cocklebur	Short	Flow.	5.6	7.3	5.3	4.2	5.8	5.2	5.2	4.6	5.6	5.4	6.6	5.2
36. " Previously induced	Long	Flow.	6.3	7.1	6.2	6.0	5.0	5.3	4.8	4.1	4.3	4.8	5.8	6.2
37. Poinsettia—Too warm to flower†	Short	Non-F.	10.3	10.2	9.3	10.6	9.0	8.1	7.4	8.3	8.8	8.8	9.1	9.9
38. "	Long	Non-F.	11.2	11.7	11.1	11.0	12.1	14.6	15.0	13.3	12.1	11.7	11.2	10.9
39. Poinsettia branches	Short	Flow.	7.3	4.3	4.8	7.0	4.8	4.9	5.2	6.1	4.7	3.7	4.9	6.7
40. "	Long	Non-F.	6.9	7.2	6.4	6.2	7.3	6.7	5.6	4.9	5.8	6.3	?	6.8
41. Perilla branches	Short	Flow.	11.1	9.4	6.7	10.8	6.8	7.7	6.3	7.3	10.0	6.8	8.4	9.3
42. "	Long	Non-F.	12.7	9.5	?	8.4	8.8	8.6	8.5	7.8	8.8	8.5	10.4	10.9
43. Nicandra branches‡	Short	Flow.	7.7	6.7	6.4	7.5	7.3	7.8	6.3	7.2	6.5	5.6	7.2	7.6
44. "	Long	Flow.	4.0	5.1	7.0	7.2	7.8	6.7	5.1	6.2	7.0	5.1	7.8	5.7

* Figure 3A. ** Figure 3C. † Figure 3B. ‡ Figure 3D.

carotene content of various samples collected throughout the 24 hours of the day are shown in table I. The significance of this phenomenon is considered in the discussion.

At first glance, it could be concluded from figure 3A, that there was a striking relation between carotene content and flowering as shown by Klondyke cosmos. A further inspection of the data reveals that the long-day plant, Penstemon, has unlike carotene curves in non-flowering and flowering plants, but the trend is opposite to that of cosmos. Jimson weed (*Datura Stramonium*) had very similar graphs for both the flowering and non-flowering plants. These results and others among the pigment data indicated no consistent relation of the carotene pigment to sexual reproduction.

The dissimilarity of the carotene graphs of two flowering plants of the day-neutral Nicandra shows the content of this pigment was related to the photoperiodic environment rather than to flowering. Two non-flowering plants of poinsettia (*Euphorbia pulcherrima*) growing at different photoperiods had unlike carotene graphs (Fig. 3B). The short day plant was kept non-flowering by a warm temperature (**6**). Two flowering plants of Xanthium had a dissimilar carotene cycle caused by different photoperiods. The plants in long days were reproductive because of an earlier induction in short days. The carotene graphs of two branches of single plants of poinsettia and of Perilla are unlike, particularly for the latter. That this situation is associated with the long and short day environment which was provided for the two branches rather than with the flowering condition is further shown by the unlike content of branches of Nicandra, although both are flowering (Fig. 3D). The carotene graphs of the flowering plants were more irregular than for the comparable non-flowering plants in more than 70 per cent. of the species sampled (Fig. 3C).

Discussion

Limited time necessitated the use of the rapid chromatographic method of measuring pigments during the present study. The consistent nature of the data and a comparison of the similar results with colorometric determinations give clear evidence that the carotene content was not uniformly related to blossoming.

If more exacting measurements of the "role of carotene content to plant performance" are desired, the question of when to sample the plants needs to be solved since it now is seen that some species have a marked diurnal cycle of carotene content, at least in some environmental conditions. Also, sampling should not be limited to a single species as different kinds of plants have typically unlike contents. Sampling should at least be done on representative species of long-day, short-day and day-neutral types. A positive correlation between carotene content and flowering might be considered as demonstrated if analyses were limited to day-time measurement of this pigment for a single species such as *Cosmos sulphureus* (**1**), *Datura Stramon-*

ium, Euphorbia pulcherrima, Nicotiana tabacum var. Maryland Mammoth, *Penstemon Hort.* sp. var. Garnet, *Xanthium echinatum,* and probably *Glycine Max* var. Biloxi (**1**). These plants would not yield a consistent correlation, however, as some would have more carotene and others would have less in the flowering plants. A positive correlation becomes even more difficult or impossible if plants of a single species, which are both flowering or both non-flowering (Fig. 3B) but growing in different photoperiods were to be used for sampling. If sampling is also done at night, the data secured are less likely to be interpreted as indicating that flowering is correlated with carotene content.

A negative or varying relation between carotene content and flowering would be expected if the following plants should be sampled: *Cannabis sativa, Chrysanthemum frutescens, Helianthus annuus, H. Tuberosus, Nicandra physalodes, Nicotiana rustica, Perilla nankinensis, Trifolium repens* var. Ladino, and *Vicia villosa.* The more irregular diurnal graphs of carotene for flowering plants than for non-flowering plants is comparable to the irregular CO_2 exchange (**5**) and irregular growth rate (**2**) previously reported for blossoming plants. The significance of these phenomena is not known, but the general similarity of the reactions of flowering plants indicates a common physiological relation to blossom induction, quite independent of the environmental conditions needed for induction.

Summary

1. The chromatographic method of pigment measurements was used, 1086 graphs being developed in the course of a study on correlations between pigments and blossoming in seed plants.

2. Different species have characteristically different amounts of the different pigments.

3. The pigment content is affected by cultural conditions, as photoperiod and temperature.

4. The pigment content, particularly for carotene and generally chlorophyll, also has a marked diurnal cycle.

5. The diurnal carotene cycle varies appreciably for branches of a single plant when these are grown in different photoperiods.

6. None of the pigment fractions showed a consistent association with flowering.

7. A positive correlation between carotene content and flowering might be indicated if the sampling was confined to the day time and only one species was sampled. Whether the amount was more or less in the flowering plant would depend upon the species. Negative correlations would be secured if other species were sampled.

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PROTOPLASMIC CHANGES DURING NUCLEAR DIVISION IN POLLEN MOTHER-CELLS AND MICROSPORES OF *TRILLIUM ERECTUM*

HERBERT STERN

(WITH FOUR FIGURES)

Received September 1, 1947

Introduction

Interest in the pollen mother-cells and microspores of Trillium arises from the fact that their rate of nuclear division is extremely slow. A complete meiotic and one mitotic division extend for five to six months, making easily possible a study of a large number of physiological properties at successive stages in the development of the meiotic or mitotic cycles. From this standpoint and with this kind of material, however, surprisingly few such studies have been made; of these, viscosity, refractive index, and protoplasmic pH, have been the chief topics of investigation (**1, 10**). *In vivo* studies of pollen mother-cells are, indeed, many; but they are mainly concerned with protoplasmic properties as such, and not with the dynamic aspect of the changes these properties undergo during nuclear division.

Just which properties to study advantageously is largely guesswork, since it is difficult to predict the factors most probably significant to the promotion of nuclear division. The present report, therefore, represents some exploratory work and deals with the behavior of pollen mother-cells and microspores in sucrose media. Two aspects of this behavior were followed, in relation to the meiotic and mitotic cycles; the viability of the cells in sucrose media, which for convenience will be referred to as their "stability," and the permeability of the cells to sucrose.

Methods

Plants of *Trillium erectum* (L.) were collected in the early fall in the vicinity of Montreal and stored in flats of 2° C. Under such conditions, the pollen mother-cells entered into active meiosis some time in December, and the microspores as the products of meiosis, underwent mitosis in April. Since all buds were to some extent synchronized with respect to stage of division, pollen mother-cells and microspores were obtained at various stages of division by removing anthers periodically from the plants.

For observation, the cells were squeezed out of the anther into a hanging droplet of the desired solution contained in a moist chamber. The latter consisted of a glass ring, 25 mm. in diameter and 6 mm. deep, fastened to a microscope slide by De Khotinsky cement. The cover-slip, from which the droplet was suspended, was sealed to the ring with petroleum jelly. Thus arranged, evaporation from the droplet appeared to be negligible, and observations could be extended for several hours or days.

Results

Stability

1. The role of sucrose in maintaining viability.—Pollen mother-cells which were transferred from an anther to a droplet of water (distilled or tap) coagulated immediately, or nearly so. As to the cause of this behavior, since no expansion but rather a contraction, of the cells was observed, a mechanical rupture of the membrane with a consequent loss of intra-cellular solutes was most unlikely. Rapid escape of intra-cellular solutes appeared to be the likeliest cause since it will later be shown that permeability of the pollen mother-cells was extraordinarily high.

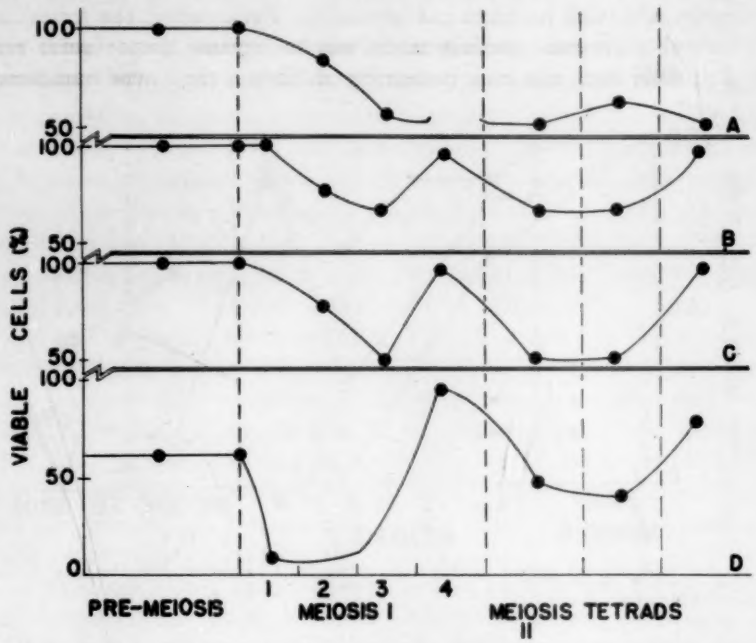

Fig. 1. Viability of pollen mother-cells in media of sucrose. a: 1.2–1.0 M; b: 0.8–0.5 M; c: 0.2–0.1 M; d: 0.08–0.05 M. Determinations made within one hour after immersion of cells in medium. Due to heterogeneity of stages in a preparation the cells in first division were grouped broadly as follows: 1. leptotene and earlier; 2. leptotene-metaphase; 3. metaphase and stages earlier than metaphase; 4. mainly metaphase and anaphase. Concentrations of sucrose are volume molar.

Coagulation was not prevented by the presence of $CaCl_2$, NaCl, nor a mixture of the two salts, over a wide range of concentrations (0.2–1.0 M). They did retard the reaction, but in general they quickly brought about a considerable increase in refractivity of the protoplasm. The latter behavior was usually associated with injury, as demonstrated by the use of neutral red and by micromanipulation. In solutions of sucrose, on the other hand, the pollen mother-cells remained viable (other sugars were not tried). In fact, where it was possible to avoid contamination by micro-

organisms, pre-meiotic pollen mother-cells could be maintained in sucrose media for three to four weeks. From observations to be described, it was clear that sucrose did not decrease the permeability of the cells. Sucrose must have had, therefore, some stabilizing effect on the protoplasm, enough to compensate for the presumed loss of solutes. In view of the rapidity of coagulation, the influence of nutritional factors in these phenomena may be ruled out. The effect of sucrose must have been of a physico-chemical nature.

The effectiveness of sucrose was found to depend partly upon its concentration. In concentrations below 0.05 M, few if any cells survived; at concentrations above 0.5 M, survival was best. The curves (fig. 1) indicate this clearly, although perhaps not strongly. Frequently, the initial effect of unfavorable sucrose media was to increase protoplasmic refractivity; since such cells were not clearly moribund, they were considered for pur-

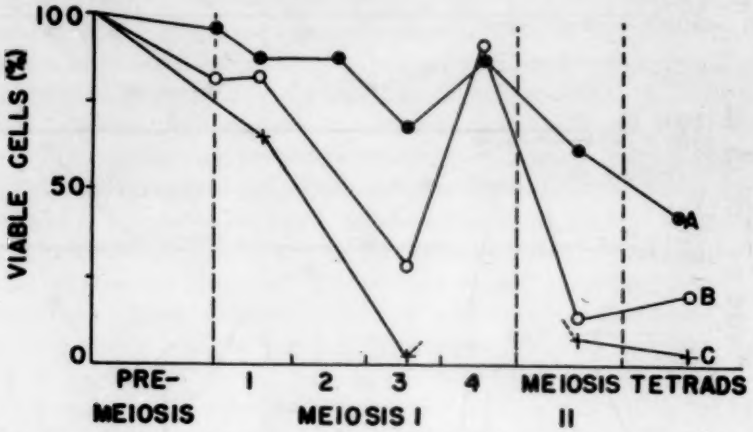

FIG. 2. Viability of pollen mother-cells in media of sucrose after twenty hours immersion of the cells.

poses of computation as "50% viable." If preparations were left over night and then examined, the moribund cells could be more easily distinguished and differences were therefore more marked, as appears in figure 2.

2. EFFECTS OF SUCROSE IN RELATION TO NUCLEAR DIVISION.—The facts of sucrose behavior might in themselves be chiefly of interest to protein chemistry. Sucrose has been reported by several investigators to have a stabilizing effect on protein solutions *in vitro* (**6, 7**), and possibly, analogous reactions occurred in the pollen mother-cells. The second aspect of this phenomenon is, however, of more direct interest to cell physiology; for the pollen mother-cells did not react similarly to sucrose at all stages of meiosis. Most striking was the presence of a variable proportion of moribund cells when pollen mother-cells in active meiosis were suspended in sucrose media. The pattern of behavior is illustrated in figures 1 and 2.

Pre-meiotic cells were in all cases associated with a high degree of stability. With the onset of active division, even if only in a small propor-

tion of the cells in an anther, preparations contained a variable percentage of moribund cells, and generally, there was an increase in refractivity of the protoplasm. With the progress of division there was a sharp decrease in percentage of viable cells, although there appeared to be an increase towards the end of Meiosis I. All cells undergoing Meiosis II were grouped together, their average percentage of viability being low. As for the quartets of microspores, the "tetrads," a fair estimate is not here given, since injury in these often occurred by the formation of hyaline vesicles at their surface (**2**). Microspores examined some time after completion of meiosis all appeared viable in sucrose media; no marked changes in stability in relation to their mitotic cycle were observed.

Permeability

1. Pollen mother-cells.—At the pre-meiosis stage all pollen mother-cells appeared to be freely permeable to sucrose. Over a broad range of concentrations (up to 1.2 M) no plasmolyses of the cells were observed, except in cases of injury where the protoplasts shrank. Dilution of the medium, as previously pointed out, produced no expansion of the cells, confirming the conclusion of a lack of semi-permeability with respect to sucrose and many intra-cellular solutes. The free penetration of sucrose was also to be inferred from its effects on the viability of the cells.

Salts also appeared to penetrate. Though somewhat difficult to demonstrate in the pollen mother-cells of Trillium, in those of Tradescantia marked increases in refractivity of the protoplasm were produced by adding small amounts of salts to a 0.2 M sucrose medium. The chlorides of Ca, Sr, and Ba were effective at concentrations in the order of 0.005 M; those of Al and La, in the order of 0.0001 M. Since the changes occurred within ten minutes after immersion of the cells, it may be concluded that the salts penetrated fairly rapidly.

The Trillium pollen mother-cells were also found permeable to members of the sulfonthalein group of dyes (Brom Cresol Green, Thymol Blue, Methyl Red, etc.). In the presence of sucrose these dyes penetrated at an appreciable rate. It was easy, incidentally, to distinguish injured cells by means of these dyes since such cells, unlike healthy ones, took on a relatively intense color.

The most obvious difference in behavior between preparations of pollen mother-cells in pre-meiosis and those in meiosis containing at least a small percentage of cells in active division, was the presence among the latter of a number of plasmolyzed cells. These cells were uninjured and could be easily deplasmolyzed by addition of water to the medium. The proportion of plasmolyzed cells varied widely in different preparations; in some cases 100% of the cells were plasmolyzed, while in others, no more than 10% were thus affected. The behavior suggested large fluctuations during meiosis, but these were not followed here.

For meiosis, only the qualitative difference was considered. A semi-

quantitative representation is found in figure 3. In this, however, only an average for each group is given, so that the fluctuations referred to are not shown. In any case, the large proportion of injured cells made a true representation difficult.

Broadly then, the behavior was as follows; free permeability to sucrose preceding meiosis, a drop in permeability of undetermined magnitude with the onset of active division, and ultimately, with the formation of microspores, a return to semi-permeability with respect to sucrose. This behavior would appear to hold true whatever the changes in osmotic pressure, although some evidence indicated a drop in osmotic pressure with meiotic development. The change may account for the larger percentage of plasmolyzed cells during Meiosis II than during Meiosis I.

2. Microspores.—The microspores, when examined about two weeks before active mitotic division of the nucleus began, were found to be poorly

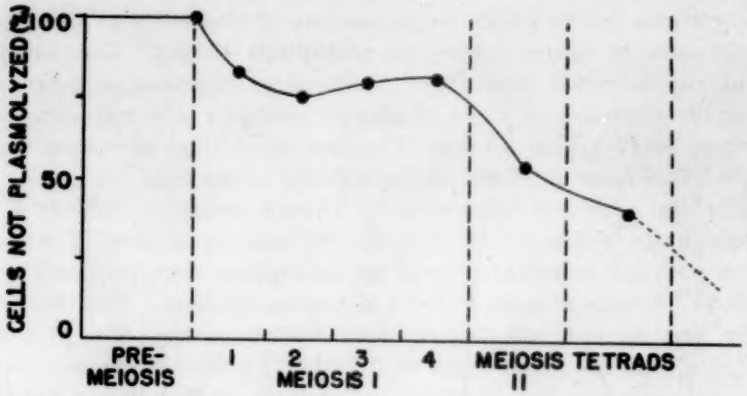

Fig. 3. Plasmolysis of pollen mother-cells in 1.2–1.0 M solutions of sucrose. Counts made within one hour after immersion of the cells.

permeable to commonly used plasmolytes. Both urea and glycerol produced plasmolysis. In a twice-isotonic solution of urea deplasmolysis-time for 100% of the cells varied from four to fifteen minutes. No deplasmolysis was observed when the cells were maintained in solutions of sucrose or glucose. Before the onset of active division, however, the cells became permeable to glucose. The major changes in permeability occurred in those preparations of microspores containing a small fraction of cells with actively dividing nuclei. Here, furthermore, changes could be better followed than in meiosis, since few cells were injured and deplasmolysis times could be readily measured. The deplasmolysis of the microspores in hypertonic solutions of glucose or sucrose was thus further confirmation of the penetrability of these substances into the cells at certain phases of development.

As in the case of the pollen mother-cells, during active mitosis in the microspores, there was a distribution of the cells in an anther among some

or all of the stages of division. The stage of individual cells could not be determined *in vivo*, so that deplasmolysis times were assigned to the preparation as a whole. To characterize each preparation in terms of percentage of cells in active division would be unsatisfactory, since that value would not take account of the proportion of cells yet to undergo mitosis, nor of those already having undergone it. Thus, a series of increasing numbers: 0, 1, 2, 3 was assigned to pre-mitosis, prophase, metaphase-anaphase, and bi-nucleated stages respectively; and the products of these numbers times the percentage frequency of the corresponding stages in the preparation was summed. For example:

(Total = 101)	Pre-mitosis	Prophase	Met-Anaphase	Bi-nucleated
Frequency %	39	38	6	17
Number × %	0	38	12	51

Thus, the larger the proportion of cells in more advanced stages of division, the larger the total. When all cells are bi-nucleated the total is 300. Plotted in this way, the deplasmolysis times indicated the general trend in permeability with development of mitosis in the microspores.

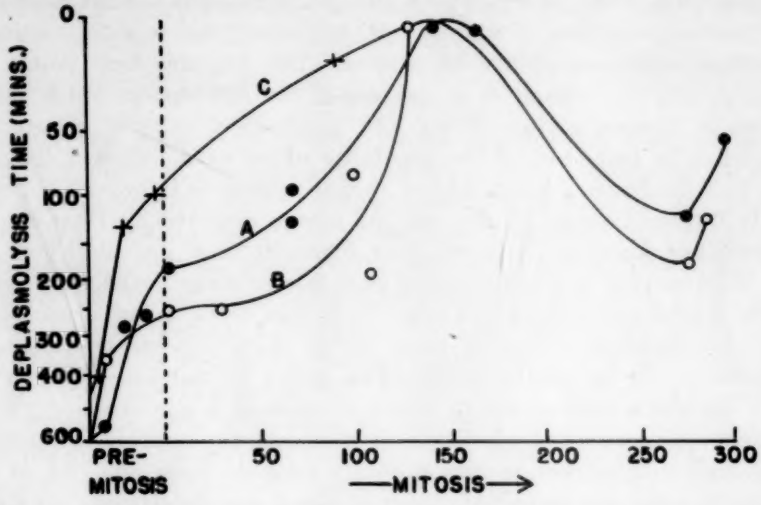

FIG. 4. Deplasmolysis of microspores in media of sucrose and glucose. a: 1.0 M sucrose; b: 1.5 M sucrose; c: 1.25 M glucose. For further explanation see text.

Rough estimates of osmotic measures determined by the method of incipient plasmolysis indicated a drop from 23 to 21 atmospheres during mitosis. The curve (fig. 4) if corrected for this would show little change in trend. A major change in osmotic pressure appeared to precede active division, the value increasing from 12 to 23 atmospheres. The change, however, has not been sufficiently studied. In the microspores then, permeability begins to increase in the pre-mitotic stage, the rate of increase falling off at the time active division begins in some cells of the anther. What stage represents the point of highest permeability cannot be inferred,

but the prophase of mitosis seems likeliest. In this respect, the behavior of the microspores differs from that of the pollen mother-cells, since in the latter, maximum permeability preceded active nuclear division.

Discussion

GENERALITY OF CHANGES.—The fact that pollen mother-cells of Trillium are somewhat "abnormal" in sucrose media was pointed out by SHIMAKURA (10), but he did not follow the phenomenon from the standpoint of meiotic development. The only references familiar to the writer which deal with a behavior similar to that designated here as "stability" are reports on studies of some marine eggs. LYON (3) long ago observed cycles of "susceptibility" to heat and cold in dividing sea-urchin eggs, and other fluctuations in susceptibility associated with cell-division in fertilized animal eggs are described by FAURE FREMIET (3). These studies, however, have not been pursued, and HEILBRUNN (4) considers the phenomenon to be merely a reflection of changes in viscosity.

Respecting permeability, references are more numerous. SHIMAKURA (9) noted that many pollen mother-cells of Tradescantia did not plasmolyze in hypertonic solutions of sucrose. He attributed this to a destruction of membrane semi-permeability by sucrose. The evidence here points differently, and very clearly so in the case of the microspores which deplasmolyze in sucrose media. KUHN (5) successfully cultured microspores only when he had reduced the possibility of outward diffusion from the cells to a minimum. Many marine animal eggs show increases in permeability during division (3, 4), and the curves obtained by HERLANT (3) for fertilized sea-urchin eggs are quite similar to those described here (fig. 4). With respect to marine animal eggs, however, the evidence is equivocal (4); although permeability changes, if they occur in rapid succession, might be somewhat difficult to detect by the techniques employed.

SIGNIFICANCE OF THE CHANGES.—The rather extraordinary susceptibility of the pollen mother-cells to injury in artificial media can be explained by their comparatively high degree of permeability; insofar, therefore, as differences with respect to this property between these and, let us say, vegetative cells are concerned, no conclusions can be drawn. Probably, the greater stability of the microspores in sucrose media is in part due to their lower degree of permeability. This explanation, however, is inadequate for the changes in stability observed *during* the meiotic cycle. Here, at leptotene when permeability is highest, stability is greatest. Loss of intra-cellular solutes alone cannot explain the increased lability of the protoplasm in presence of sucrose following the onset of active meiosis. The seat of the difference must be in the protoplasmic substrate itself, and here the properties of the protein fraction are probably most important. As to the nature of the change, the question is very much open. A causal relation, if any, between nuclear division and lability of protoplasmic proteins is a problem yet to be studied. Thus far, only the theory of RAPKINE

(**8**), that denaturation of cellular proteins exposes the –SH groups necessary to division, lends support to the idea.

A general discussion of the significance of permeability to nuclear division has already been published (**12**). Whatever significance may be attached, it is most improbable that a change in degree of permeability would be a causal factor in nuclear division. The changes in passive permeability are more likely the results of more fundamental processes which cells in division undergo. That which appears to be common to the phenomena here observed is the existence of a marked difference in properties between cells in pre-division and those in active division. Not only stability and permeability, but current studies on respiratory behavior by the writer indicate much the same pattern, namely a fairly sharp change in behavior from the pre-division to the active division state. It would seem difficult, then, to embrace entirely the physiological mechanism of nuclear division by a study of properties of proliferating tissues in general. A crucial point of study appears to be the complex of changes occurring prior to or coincident with active morphological division. The changes here described may be best regarded as reflections of more deeply seated phenomena which must be resolved by further research.

Summary

A study was made of the behavior of the pollen mother-cells and microspores of Trillium in sucrose media with respect to the following: 1. their viability (referred to as their "stability") in relation to the cycle of nuclear division and 2. their permeability in relation to the cycle of division.

In sucrose media the pollen mother-cells were found to be stable preceding meiosis, but with the onset of active meiosis a drop in stability occurred. The microspores appeared to be stable in sucrose media. The effect of sucrose on the protoplasm was considered to be physico-chemical, and the probability of some change in property of the protoplasmic substrate coincident with active division was discussed.

In pre-meiosis the pollen mother-cells were freely permeable to sucrose. With active division there occurred a drop in degree of permeability. The microspores, some time after completion of meiosis, were impermeable to glucose and sucrose. With the onset of active mitosis their permeability increased. Maximum permeability appeared to be reached during the prophase of mitosis.

The writer wishes to express his thanks to Professors G. W. Scarth and C. L. Huskins for supervision of his work; and to the National Research Council of Canada, under which the writer held two studentships (1941–'43), for its financial assistance.

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NOTES

Election of Officers.—The following have been elected as new officers of the American Society of Plant Physiologists: President, D. B. Anderson; Vice-president, James Bonner; Executive Committee, B. S. Meyer; Editorial Board, W. E. Loomis. The terms of office for the president and vice-president are for one year. Members of the executive committee and editorial board are elected for three-year terms.

Meeting of Plant Physiologists.—The twenty-third annual meeting of the American Society of Plant Physiologists will be held in Cincinnati, Ohio, Wednesday, Thursday, and Friday, September 8, 9 and 10, 1948. The program committee for this Cincinnati meeting consists of the Secretary, as ex-officio chairman, Dr. H. J. Kersten of the University of Cincinnati, and Dr. F. M. Turrell of the University of California. The American Society for Horticultural Science is also meeting in Cincinnati at the same time as the American Society of Plant Physiologists. A.S.P.P. headquarters will be in Hotel Sinton, and the Horticulturists will be in Hotel Gibson nearby.

Professor Dmitrij N. Pryannishnikov.—The death of Professor Pryannishnikov, 83, one of Russia's leading agricultural scientists, was announced in a Tass dispatch from Moscow, dated May 3, 1948. Pryannishnikov trained many prominent agronomists, 40 of whom are now professors, and was the author of 360 scientific works. He was a member of the Academy of the U.S.S.R. He became a member of the American Society of Plant Physiologists in 1936.

Illini Plant Physiologists.—Plant Physiologists at the University of Illinois sponsored a lecture at Urbana by Dr. F. C. Steward, Visiting Professor of Botany, University of Rochester, and Professor of Botany, Birbeck College, University of London, on April 29, 1948. Dr. Steward spoke on "Metabolism and salt uptake with special reference to the potato tuber."

New England Section.—The annual session of the New England Section of the A.S.P.P. took place at the University of Massachusetts, Amherst, May 21–22. Nineteen papers were presented by the group from 15 institutions with a total of 60 registering. An afternoon tea, a banquet, and business meetings of the executive committee and representatives were diversions. The officers for the ensuing year are: Chairman, Dr. T. G. Phillips, University of New Hampshire; Vice-Chairman, Dr. R. H. Wallace, University of Connecticut; Secretary-Treasurer, Dr. L. H. Jones, University of Massachusetts.

Physiologica Plantarum, Volume I.—HANS BURSTRÖM, Editor-in-chief. Publisher: Einar Munksgaard, 6 Norregade, Copenhagen, Denmark. 1948. 40 Danish Kroner.

The first number of this new quarterly periodical, published by the Scandinavian Society for Plant Physiology, covering all branches of plant physiology, has recently become available. In addition to Professor Burström, the consulting editors are Professor R. Collander, Helsinki, Finland; Dr. E. K. Gabrielsen, Copenhagen, Denmark; and Professor O. Hagem, Bergen, Norway. Physiologia Plantarum has been inaugurated in order to promote the plant physiological research in the Scandinavian countries and as a means of presenting its results to interested scientists all over the world. The journal is open to contributions from Scandinavian and foreign members of the Society, and the papers are printed in English, French, or German. The journal thus wishes to serve also as an international organ for scientific information within the field of plant physiology. Physiologia Plantarum is issued quarterly, each number containing about 100 pages. Subscriptions can be placed with Einar Munksgaard, 6 Norregade, Copenhagen or any bookseller all over the world.

Contents of the first number are: Glavind, J. and Dam, H. (Copenhagen): Factors influencing the growth of Johne's bacillus; Gabrielsen, E. K. (Copenhagen): Effects of different chlorophyll concentrations on photosynthesis in foliage leaves; Pehrson, S. O. (Stockholm): Studies on the growth physiology of *Phacidium infestans* Karst; Burström, H. (Lund): A theoretical interpretation of the turgor pressure; Algèus, S. (Lund): Glycocoll as a source of nitrogen for *Scenedesmus obliques;* Saubert-v. Hausen, S. (Helsinki): On the role of growth substances in higher plants; Boysen Jensen, P. (Copenhagen): Formation of galls by *Mikiola fagi;* Melin, E. and Mikola, P. (Uppsala): Effects of some amino acids on the growth of *Cenococcum graniforme.*

Forest Soils.—HAROLD J. LUTZ and ROBERT F. CHANDLER. John Wiley & Sons, 440 Fourth Avenue, New York 16. 514 pages, 1946. $5.25.

This book covers the fundamentals of soil science with applications to forestry suitable for use in the course in soils required of forestry students in American colleges and universities. It also serves as a source of reference to reports on the more important researches on forest soils. The contents and sequences of subject matter are indicated by the chapter headings: Introductory; Soil-forming Minerals; Soil-forming Rocks; Distintegration and Decomposition of Minerals and Rocks; Forest-soil Organisms; The Organic Matter of Forest Soils; Nature and Properties of Soil Colloids; General Physical Properties of Forest Soils; The Water Relations of Soils, Particularly Forest Soils; General Chemical Properties of Forest Soils; Soil Formation; Forest Soil Classification; Soil Erosion and Forest-soil Deterioration. Botanists, foresters, ecologists, and soil scientists will find this work a valuable source of forest soils knowledge. The extensive list

of references given at the end of each chapter and the advanced and excellent nature of some of the discussions also make this a very useful reference book.

Diseases of Field Crops.—JAMES G. DICKSON. McGraw-Hill Book Company, 330 West 42nd Street, New York 18. 429 pages, 1947. $4.50.

The book is a useful reference on current information concerning diseases of field crops, including barley, corn, millet, oats, rice, rye, sorghum, Sudan grass, Johnson grass, sugarcane, wheat, forage grasses, alfalfa, sweetclover, clovers, soybeans, cotton, flax, and tobacco. A brief introduction is followed by a chapter on physiological anatomy of plant groups in relation to disease. A rather extended, but selective reference list is appended to each chapter. Although the diseases are discussed in the main body of the text primarily from the standpoint of the particular crop affected, they are regrouped in the appendix on the basis of the primary causal factor, a suggested list for class presentation on this basis and also a list of the bacteria and fungi arranged by order and family. A comprehensive subject matter index enhances the usefulness of the book, both for the student and the research worker. The book is well written and it will serve as a condensed reference for plant pathologists and agronomists.

Basic Botany.—FRED W. EMERSON. Blakiston Company, 1012 Walnut Street, Philadelphia 5, Pennsylvania. 372 pages, 1947. $4.00.

This book is a text for an introductory course in botany. As stated in the preface, physiology, anatomy, morphology, taxonomy, genetics, and ecology are all included, but not in the traditional order. Leaves, stems, and roots are discussed in detail as integral parts of the plant, which is portrayed as a functional as well as a morphologic unit. Plants with which the student is apt to be familiar have been chosen for illustrative material. The text gives considerable attention to the scientific approach in solution of botanical problems. The illustrations are new and original. The scope is the usual one of morphology and physiology with chapters devoted to genetics and evolution, plus excellent sections on plant classification, ecology, and conservation.

Chemical Insect Attractants and Repellents.—VINCENT G. DETHIER. Blakiston Company, 1012 Walnut Street, Philadelphia 5, Pennsylvania. 289 pages, 1947. $5.00.

The book is primarily for use by entomologists and biochemists. The author describes many physical and chemical characters of plants that act as repellents. Some of these are of genetic origin and hence could be used in attempts to breed varieties with considerable resistance to certain serious insect pests. It contains a comprehensive bibliography at the end of each chapter. The author states that he has made "an attempt to bridge the borderline between chemoreception and the broader aspects of 'insect' be-

havior based upon it." The book represents a theoretical study and "it is neither a compilation of recipes for attractant and repellant substances nor a manual on insect control," but it contains much practical information and some recipes for baits that should be of value to many investigators. The book is well written and generously illustrated.

Two Blades of Grass: A History of Scientific Development in the U. S. Department of Agriculture.—T. SWANN HARDING. University of Oklahoma Press, Norman, Oklahoma. 352 pages, 1947. $3.50.

This book is a history of the U. S. Department of Agriculture. The author came to the Department in 1910, and for many years was editor of scientific publications in the Office of Information, giving him an authorative knowledge of his subject. The book is also a history of agricultural research in America, because in one way or another scientists of the Department of Agriculture were associated with most investigations whether done at the state experiment stations or in and around Washington, D. C.

Annual Review of Microbiology, Volume I.—C. E. CLIFTON, editor. Annual Reviews Incorporated, Stanford, California. 404 pages, 1947. $6.00.

This new review journal envisages frequent and more extensive and critical summaries of active phases of research in microbiology than are now available in other abstract periodicals. Reviews of the same subject are to be made by different authors in succeeding years to insure variations in approach and emphasis. Chapters in the first volume comprise: Morphology and cytology of protozoa, Antigenic variations in protozoa and bacteria, Variations in phytopathogenic fungi, Life cycle of malarial parasites, Growth factors for protozoa, Bacterial metabolism, Nitrogen metabolism, Industrial fermentations, Quaternary ammonium compounds, Antibiotics, Chemotherapeutic agents, Immunochemistry, Enteric infection, Rickettsiae, and Respiratory viruses. Each section carries a comprehensive bibliography. Complete author and subject indices facilitate rapid reference use.

Bibliography of References to the Literature on the Minor Elements: Fourth Edition. Chilean Nitrate Education Bureau, Incorporated, 120 Broadway, New York 5, New York.

The Chilean Nitrate Educational Bureau, Inc. announces publication of the Fourth Edition of the Bibliography of References to the Literature on the Minor Elements and Their Relation to Plant and Animal Nutrition. The first edition of this bibliography was published in August, 1935; the second, in November, 1936; and the third, in February, 1939. Subsequently, the 1st Supplement was published in April, 1940; the 2nd, April, 1941; the 3rd, April, 1942; the 4th, June, 1943; the 5th, July, 1944; the 6th,

November, 1945; and the 7th, July, 1947. This Edition includes all material published in the Third Edition and the seven supplements thereto, together with some new material, making it complete as of June 30, 1947. There are 10,000 abstracts, which include 206 crops, 45 elements and 4,463 authors. Complete indices are provided, i.e., Author, Element, Botanical and General Nutrition.

The Study of Plant Communities. HENRY J. OOSTING. W. H. Freeman and Company, 549 Market Street, San Francisco 5, California. 1948. 381 pages. $4.50.

This informative book is the outcome of an introductory course in plant ecology. It is written from the synecological point of view in sympathy with present-day physiological thinking. The author has greatly enriched the text by drawing upon autecology, taxonomy, morphology, historical and paleobotanical materials as well as those of the allied sciences. He has employed the field-work perspective as the one the student finds most easily understandable. Original illustrations occur in generous numbers.

The aim of the book is to describe the characteristics and variations of plant communities, the methods by which they can be distinguished and described. This approach naturally leads into a discussion of the factors that govern communities regionally and locally. The scope of plant community dynamics is deftly indicated, spreading from rudimentary bionomic phenomena to its impact on the economy of a nation and on the "status quo" of man. A section is devoted to the probable permanence of climax, including a study of past climaxes and the measures required to maintain them. The potentialities of ecology in practical considerations are outlined through a survey of desirable applications in range management, agriculture, conservation, landscaping, forestry and human relations. Comparative examples are taken from many parts of the world but the major stress is placed on the ecology of the North American continent. The book contains the following parts: Introduction, The plant community, Factors controlling the plant community, Community dynamics and practical considerations.